Certificate

This is to certify that the work entitled **"TO STUDY THE EXTENDED CLINICOLABORATORY PROFILE OF THE MALE PARTNER OF INFERTILITY IN BUNDELKHAND REGION"** which is being submitted as a thesis for M.D. (Medicine) examination, 2000 of Bundelkhand University by Dr. Munish Kumar Sachdeva, has been carried out in the Department of Medicine, M.L.B. Medical College, Jhansi.

He has put in the necessary stay in the department as per university regulations.

(R.C. Arora)
M.D., D.Sc.
Professer and Head,
Department of Medicine,
M.L.B. Medical College,
Jhansi
(Guide)

Dated :

Certificate

This is to certify that the work entitled **"TO STUDY THE EXTENDED CLINICOLABORATORY PROFILE OF THE MALE PARTNER OF INFERTILITY IN BUNDELKHAND REGION"** has been carried out by Dr. Munish Kumar Sachdeva under my direct supervision and guidance. The techniques and statistical methods used in this thesis have been undertaken by the candidate himself and checked by me from time to time.

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Dated :

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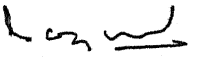

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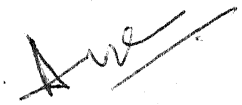

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Acknowledgement

Words and lexicons cannot do full justice in expressing my reverence and profound gratitude to Dr. R.C. Arora M.D., D.Sc. Professor and Head, Department of Medicine M.L.B. Medical College,Jhansi, my esteemed teacher under whose benevolence and able guidance, I read, learned and ventured to write. Still I take this opportunity to acknowledge most humbly from the inner recess of my heart and with a deep sense of gratitude my indebtness to him. His inspiring benefaction bestowed upon me so generously helped me to carry out this persent work. My worshipful adorations to him.

It is indeed a great privilege and immense pleasure for me to record my deep sense of gratitude and heartfelt thanks to Dr. (Smt.) Sunita Arora, M.S., Associate Professor, Department of Gynaecology, who bestowed upon me knowledge, time to time during this study and made it possible to complete this work.

In no lesser degree and with overwhelming gratitude, I express my sincere most thanks to my co-guide Dr. R.K. Agarwal, M.D., Professor and head Department of microbiology M.L.B. Medical College, Jhansi. His fatherly attitude, invaluble suggestions enabled my to take over the difficulties during the period of study.

I find myself highly indebted to my co-guide Prof. A.K. Gupta M.D. Department of Radiology, for his expert guidance, invaluable advice and unstinting help at every juncture.

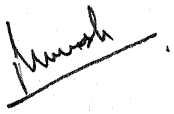
I have no dearth of feelings, but an understanding of the futility of my expression as I try to bring forward my sincere gratitude to my teachers Dr. P.K. Jain, M.D. M.N.A.M.S. Dr. Praveen Jain M.D., D.M. and Dr. Navneet Agarwal, M.D. for their unexhaustable, sincere advise and help rendered to me throughout this study.

I feel highly obliged to my Computer operater Mr. M. Khursheed for preparing this manuscript in an exemplary manner.

Every particle of me is indebted to my parents and in laws for their love, sacrifice, care and inspiration at every moment of my life. Last and certainly not the least this study would not have been possible without the active participation of my wife Dr. (Smt.) Charu G. Sachdeva. She has always been available with ready invaluable suggestions regarding case and unending encouragement. This study carries imprints of her help, wise and meticulous advise. I am really thankful to her.

Dated :

Munish Kumar Sachdeva



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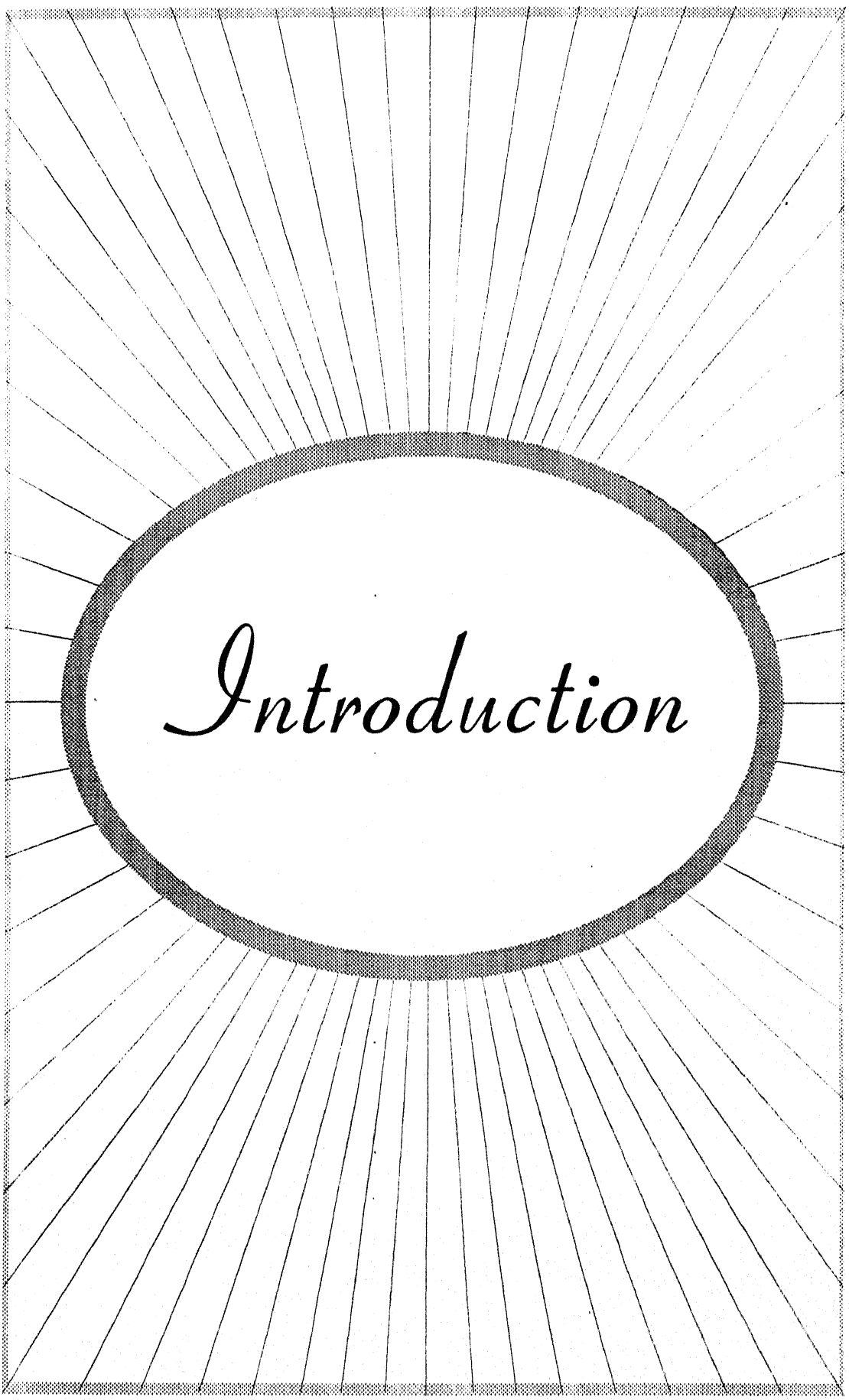

Introduction

INTRODUCTION

The inability to conceive can be a very perplexing & distressing problem for a couple. In India, approximately 15% of all married couples are involuntarily infertile[1]. Thus in reference to our country, it is easily understandable how big the problem is. Inspite of this, it is often overlooked as a serious life problem or ignored as a legitimate health issue. It was initially thought that the female partner was responsible for infertlily and were the major victims of social taboos relating infertility as a cause rather than an expression of a disease process especially in backward communites of our country but now it has been seen that 40% of infertility is wholly or in part due to male factors. Infertile couples often suffer from extreme frustration, feeling of incompetence and social isolation.

Although overpopulation is now a major concern for the world as a whole and especially for countries like India but recently the right of a couple to have a child and enjoy prosperous married life has been increasingly recognised. The infertile couple especially the female, has from the time immemorial been subjected to all kinds of stracization. This trend unfortunately still prevails, specially in the socioeconomically poor and uneducated class, which is of plenty in Bundelkhand region. It is also well recognised that more the backward the region is, the male of infertile union from that region are increasingly less inclined to be

investigated by the doctor because of a hidden fear of being declared invalid in a male dominated society, and since complete investigation of an infertile male is not only a lengthy process but also sometimes frustrating ,to reach a proper working diagnosis.

Thus, they justifiably deserve more support from the consulting physician, Patients present to the physician with extreme confidence for expert advice, diagnosis and rational therapy.

Large number of surveys and studies have been done assessing the male contribution to problem of infertility, Various studies have put the incidence from 25% to 40%. Bayertz (1967) has stated that in about 30% of infertile couples the abnormalities in husband & in next 20-30% the abnormalities lies in both partners[2].

The WHO study reported that for male factor only the male contribution was 22% in developed world; 8% in Africa & 13% in Chandigarh (India). Also when the percentage of investigated males who failed to show any cause; they were then put in the category of no demonstrable cause by WHO, these factor was reported to be 49% from developed countries, 46% from Africa & 73% from Chandigarh.

In the United Kingdom, it has been estimated that 24% of couples will consult a doctor about infertility at some stage during their lives[3]. While various surveys have put the frequency very much dependent on the definition of male &

female infertility disorders & on the thoroughness of investigations.

Infertility is also a common problem affecting at least one couple in six in Europe Epidemiologic surveys of new couples attending infertility clinics in United Kingdom have suggested that about one in four present with clear evidence of defective sperm function; abnormal semen quality being the most common single diagnosis arrived at many clinics.

Significant stress is experienced by couples during initial medical interview & at the time of diagnotics medical investgation; however questions were raised regarding sexual relationship of couples suffering from an unexplained infertility diagnosis. Thus incidence & prevalence of male infertility vary greatly from country to country & within parts of country.

The present study was conducted to study the clinico-laboratory profile in male infertility in Bundelkhand region which is very backward region both from socio-economically as well as educational point of view.


Review
of
Literature

REVIEW OF LITERATURE

There is an increasing awareness in the medical community of the fact that subinfertility in the male contributes significantly to barren marriages. It is assumed that as part of infertility i.e. when should a couple be considered as childless or even more appropriately, when should the couple be considered for investigation into possible causes of infertility.

"Methews & Duncan" originally defined infertility "if a married couple did not got any issue within 1yr. of marriage". But recent investigations points out infertility as "Inability or failure to conceive within 12 months with regular sexual intercourse". In order to be fertile, the male partner must succeed in depositing his semen intravaginally.

In is now over 300 years since "Anthoni Von Leeuwenhoek" reported his first observation of motile spermatozoa in the human ejaculate, in his dramatic letter to Real Society of November 1677 "de Natise semini genitale Animalcules"[4].

In a further letter dated 1685, he went on to speculate that the existence of spermatozoa or animalcules in semen was associated with its fertilizing ability, & moreover that infertility could be caused by either the absence of spermatozoa, or their having reduced function. Charles Bonnet, writing almost 100 years later in 1971 was less certain of role of spermatozoa in semen asking" do they so largely scattered"[4]. The first quantitative study on seminal fluid was

probably performed by "Lazzaro Spallanzani" in 1780[4], while the, while the later work of Prevost Dumas & Herwig began to elucidate the mechanism of fertilization establishing that one sperm was necessary to fertilize each egg. The direct quantitative study of human ejaculate did not begain until the early part of the present century when Benedict, working in NewYork, published a brief report on counting the spermatozoa in semen using a blood counting chamber, a early as 1902. Not until 1929 was the quantitation of spermatozoa in human semen placed on a scientific footing by Macomber & Sanders who began to evaluate the range of sperm concentritions in semen associated with fertility. This was followed by new classic work of John Macleod who undertook a range of studies of semen quality in groups of fertile & potentially infertile men[5] & in doing so laid the foundation for modern diagnositc andrology.

Even in modern times abnormal semen quality is most common investigation tool arrived in most clinics[6], thus one of the most important laboratory parameters in an infertile male is SEMEN ANALYSIS.

Male reproductive tract is an organ complex concerned with ultimate goal of the reproduction of the race. The system includes gonads, the excretory ducts, & several endocrine glands. In the adults, the testis has two main functions, spermatogenesis i.e. the production of germ cells, & steroidogenesis i.e. synthesis & secretion of sex steriod hormones. The epididymes contributes to maturation of sperm.

The sex accesory glands provide the bulk of the ejaculate[7].

Sexual differentiation, development of gonads & of the genital tract continues throughout from the time of birth to puberty; activation of endocrine hypothalamo-pituitary- gonal axis, appearence of secondary sexual characterstics, & initiation of spermatogenosis. It is only after puberty that male reproductive system is ready for reproduction.

Thus the entire male sexual development depends on a delicate interplay between anatomic, functional, & regulatory (genetic & hormonal) factors[7].

The development of the male reproductive tract results from a series of steps in an orderly fashion-the differentiation of the gonad into a testis. The initiation of testicular steroidogenesis & finally, the expression of androgenic activity in targat tissues. These three steps are genetically controlled by genes present on both X & Y chromosomes & autosomes. Pericentic region of Y chromosome is implicated in testis determination region of the Yq arm have been associated with spermatogenesis[7].

Infertility can be brought by gene mutation that causes depression or arrest of spermatogenesis in adult. In men, maturation arrest at various stages of spermatogenesis ocurrs in some individuals with apparently normal karyotypes & in men with various structural & numerical chromosomal abnormalities[28].

Probobly keeping in mind these early developments it has

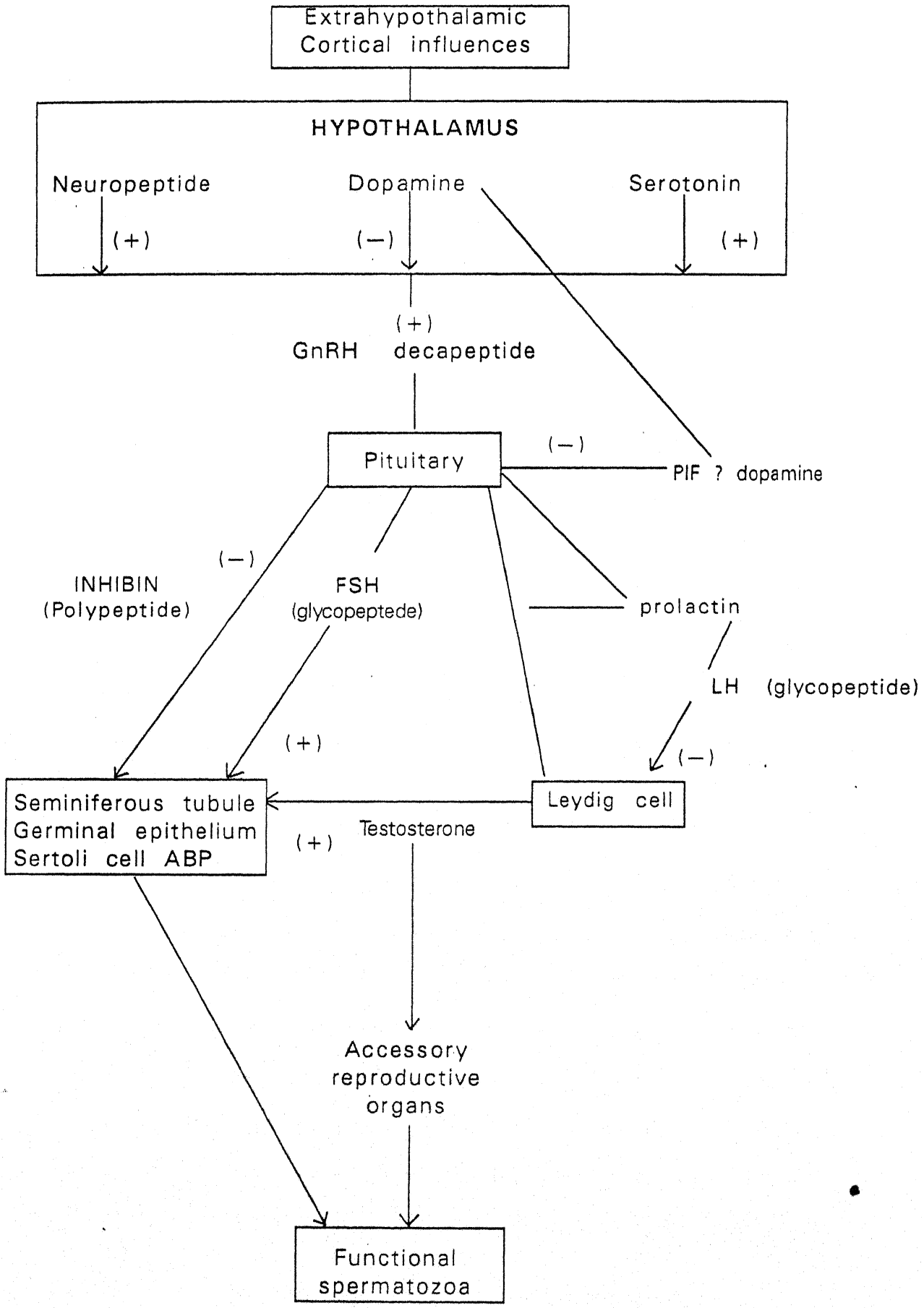
been suggested the basic laboratory investigations of any patient with infertility should include an adequate semen assessment, chromosomal studies & hormonal assessment[29,30].

Therefore, Infertility evaluation should involve a systematic approach using the most non-invasive procedures just & then to proceed for invosive & specalesed proceures (testicular biopsy) towards end of evaluation. So we will start with a brief account of the basic anatomy of male genital tract & relevant physiology.

THE HYPOTHALAMIC PITUITARY GONADAL AXIS

Steroidogenic function of the testis is gonadotropin dependent regulation of testosterone production is governed by an interplay between the hypothalamus, hypophysis & testes. The hypothalamic-pituitary-testicular axis functions in a cybernetic circle in which the role of the common circuit is played by the hypothalamus (through LHRH secretion). Pituitary secretion can be viewd as regulating the circuit & testicular secretion (sex steroids & inhibin) issue feedback signals,therefore, any disruption of the system or dysfuction may lead to abnormal sexual differeniation, sexual infantilism or infertility[7].

In normal adults, a fall in testosterone secretion results in an increased release of LH which, in turn, stimulates testosterone secretion in such a way that testosterone levels are maintained rather constant except of slight day-night


Extrahypothalamic
Cortical influences
HYPOTHALAMUS
Neuropeptide
Dopamine
Serotonin
(+)
(−)
(+)
(+)
GnRH decapeptide
Pituitary
(−)
PIF ? dopamine
(−)
INHIBIN
(Polypeptide)
FSH
(glycopeptede)
prolactin
LH (glycopeptide)
(+)
(−)
Seminiferous tubule
Germinal epithelium
Sertoli cell ABP
Leydig cell
(+)
Testosterone
Accessory
reproductive
organs
Functional
spermatozoa

varaitions and biologic rhythms. LH is secreted in successive spikes at a frequency of approximately one per hour. Negative feed back control of LH secretion is exerted by both sex steroids testosterone & E_2, but their short term effects & site of action differ. In long term, androgens diminishes the response of LH to LHRH altering little that of FSH, while estrogens potentiate both FSH & LH release. Besides E_2, another substance secreted by the testis, named inhibin, speciafically inhibits FSH,testosterone having little effect on its secretion.Inhibin is produced by sertoli cells & acts not only at pituitary level but also on the hypothalamus & the testis.

The role of prolaction in male reporduction is not well understood. Prolactin alone has little action on the male reproductive tract but it potentiates the effect of LH on the Leydig cell & that of testosterone on the prostate & seminal vesicle. Prolactin is able to increase the androgen receptor complex which is transferred to nucleus in target tissues[8].However,the mechanisms by which,the hyperprolactonemic men, lead to decreased reproductive function is unknown. Prolactin is thought to produce impotence independently of its lowering action on testosterone secretion[9].

The main action of .LH is to stimulate testosterone, a secretion by Leydig cells.FSH stimulates sertoli cells, protein synthesis ABP & inhibin secretions & the aromatization of testosterone to E2. The pulsatile pattern of gonadotropin

secretion results from pulsatile secretion of LH-RH. Gonadal & gonadotropic function undergo subsequent periods of activation & depression throughout life. It is becoming evident that the CNS mediates those "maturational changes'by modulating the synthesis & or release of LHRH[10].

THE TESTES:

Testosterone is secreted episodically from leydig cell in reponse to LH pulses & has a diurnal pattern, with peak level in the early morning & the trough level in the late afternoon or early evening. In the intact testis, LH receptors decreases or down regulate after exogenous LH administration.Testosterone; the primary inhibitor of LH secretion in males. Testosterone may be metabolized in peripheral tissue to potent androgen dehydrotestosterone or the potent estrogen estradiol.

Large doses of GnRH or its analogs can reduce the number of LH receptors & therefore inhibit LH secretion. This has been applied clinically to cause medical castration in men with prostate cancer. Estrogen inhibits some enzymes in the testosterone synthesis pathway & therefore directly affects testosterone production. There also appears to be an intra-testicular ultrashort loop feedback such that exogenous testosterone will override the effect of LH & inhibit testosterone production.In normal male only 2% of testosterone

is free. 44%bound to testosterone-estradiol binding globulin or TeBG,also called sex hormones binding globulin. 54% of testosterone is bound to albumin & other proteins. These steroid binding proteins modulate androgen action. TeBG has higher affinity for testosterone than for estradiol & changes on TeBG alter or amplify the hormonal milieu. TeBG levels are increased by estrogens, thyroid administration & cirrhosis of liver & may be decreased by growth hormone & obesity. The biological actions of androgens are exerted on the target organs that contain specific androgen rceeptor proteins. Testosterone leaves the circulation & enters the target cells where it is converted to the more potent androgon dehydrotestosterone by an enzyme 5 alpha-reductase. The major functions of androgens in target tissue include regulation of gonadotropin secretion by hypothalamic-pituitary axis, initiation & maintainence of spermatogenesis, differentiation of internal & external male genital system during fetal development & promotion of sexual maturation at puberty.

3 Seminiferous tubules

The seminiferous tubules contains all the germ cells at various stages of maturation and their supporting sertoli cells. These account for 85 to 90% of the testicular volume. Sertoli cells are a fixed population of non dividing cells. They rest on the basement membrane of the seminiferous tubules. They are linked by tight unctions. These tight junctions couplesd with the close approximation of the myoid cells of

the peritubular contractile cell lyayers serves to form the blood testes barrier. This barrier provides a unique micro-environment that facilitates spermatogenesis and maintains these germ cells in an immunologically previleged location.This isolation is important because spermatozoa are produced during puberty, long after the period of self recognition by the immune system. If these developing spermatozoa were no immunologically protected, they would recognized as foriegn and attacked by the body's immune system. Sertoli cells appear to be involved with the nourishment of the developing germ cells as well as the phagocytosis of te damaged cells. Spermatogonia and young spermatocytes are lower down in the basal compartment of the seminiferous tubules, whereas matured spermatocytes and spermatides are sequestered higher up in the adluminal compartment.The germinal cells or the spermatogenic cells are arranged in an orderly manner from the basement membrane upto the lumen. Spermatogonia lie directly on the basement membrane, and next in order, progressing upto the lumn, are found the primary spermatocytes, secondary spermatocytes and spermatids. Spermatogenesis is a complex process whereby primitive stem cells or spermatogonia, either divide to reproduce themselves for stem cell renewal or they divide to produce daughter cells that will later become spermatocytes. The spermatocytes evetually divide and give rise to mature cell lines that eventually give rise to spermatids. The spermatids then undergo a transformation into spermatozoa. This transformation

includes nuclear condensation, acrosome formation, loss of most of the cytoplasm, development of a tail and arrangement of the mitochondria into the middle Ipiece of the sperm which basically becomes the engine room to power the tail. Groups of germ cells tend to develop and pass through spermatogenesis together. This sequence of developing germ cells is called a generation. These generations of germ cells are basically in the same stage of development. There are six stages of seminiferous epithelium development. The progress from stage 1 through stage 6 constitutes 1 cycle. In humans the duration of each cycle is approx. 16 days and 4.6 cycles are required for a mature sperm to develop from early spermatogonia. Therefore, the duration of entire spermatogenic cycle in humans is 4.6 cycles times 16 days equals 74 days.

4. Hormonal control of Spermatogenesis.

An intimate structural and functional relationship exists between the two seperate compartments of the testis, i.e. the seminiferous tubule and the interstitium between the tu bule and the interstitium between the tubules.LH affects spermatogenesis indirectly in that it stimulates androgenous testosterone production. FSH targets sertoli cells. Therefore, testosterone and FSH are the hormones that are directed at the seminiferous tubule epithelium. Androgen-binding protein which is a sertoli cell product carries testosterone intracellularly and may serve as a testosterone reservoir within the semininferous tubules in addition to transporting

testosterone from the testes into the epididymal tubule. The physicial proximity of the leydig cells to the seminiferous tubules and the elaboration by the sertoli cells of the androgen-binding protein, cause a high level of testosterone to be maintained in the micro environment of the developing spermatozoa. The hormonal requirements for initiation of spermatogenesis. For spermatogenesis to be maintained like for instance after a ituitary obliteration, only testosterone is required. However, if spermatogenesis is to be re-initiated after the germinal epithelium has been allowed to regress completely, then both FSH and testosterone are required.

5. **Transport- maturation-storage of sperm.**

Although the testis is responsible for sperm production, the epididymus is intimately involved with the maturation, storage and transport of spermatooa. Testicular spermatozoa are non-motile and were felt to be incapable of fertilizing ova. Spermatozoa gain progrrssive motility and fertilizing ability after passing through the epididymis. The coiled seminiferous tubules terminate within the rete testis, which in turn coalesce to form the ductuli efferents. These ductuli efferents conduct testicular fluid and spermatozoa into the head of the epididymus. The epididymus. The epididymis divides into the head, of the epididymis. The dpididymus consists of a fragile single convoluted tubule that is 5-6 meters in length. The epididymis is divided into the head, body and tail. Although epididymal transport time varies with

age and sexual activity,the estimated transit time of spermatozoa through the epididymus in healthy males is approximately four days. It is during the period of maturation in the head and body of the epididymus that the sperm develop the increased capacity for progressive motility and also acquire the ability to penetrate oocytes during fertilization. The epididymis also serves as a reservoir or storeage area for sperm. It is estimated that the extragonadal sperm reservoir is 440 million spermatozoa and that more than 50% of these are located in the tail of the epididymis. The sperm that are stored in the tail of the epididymis enter the vas dferens which is a muscular duct 30-35 cm in length. The contents of the vas are propelled by peristaltic motion into the ejaculatory duct. Sperm are then transported to the outside of the male reporductinve tract by emission and ejaculation during emission, secretions from the seminal vesicle and prostate are deposited into the posterior urethra. Prior to ejaculation peristalsis of the vast deferens and bladder neck-occur under sympathatic nervous control. during ejaculation, the bladder neck tightens. and the external sphincter relaxes with the semen being propelled to the urethera via rhythmic contractions of the perineal and bulbourethral muscles. It is true that the first portion of the ejaculate contains a small volume of fluid from the vast deferens which is rich in sperm. The major volume of dthe seminal fluid comes from the seminal vesicles and secondarily

the prostate. The seminal vesicles provide the nourishing substrate fructose as well as prostaglandins and coagulating substrate. A recognized function of the seminal plasma is its buffering capacity on the acidic vaginal environment. The coagulum formed by the ejaculated semen liquifies within 20 to 30 munutes as a result of prostatic proteolytic enzymes. The prostate also adds zinc phospholipids, spermine, and phosphate to the seminal fluid. The first portion of the ejaculate charactistically contains most of the spermatozoa and most of the prosatic secretions, while the second is composed primarily of seminal vescicle secretions and fewer spermatozoa.

Fertilization normally takes place within the uterine tubes after ovulation has occured. During the menstual mid cycle, the cervical mucus changes to become more abundant,thinner and more watery. These changes serve to facilitate entry of the sperm into the uterus. and to protect the sperm from the highly acidic vaginal secretions. Physiologic changes in the spermatozoa know as capacitation occur within the female reproductive tracts in order for fertilization to occur. As the sperm interacts with the egg. there is initiation of new flageller movement called hyperactive motility and morphologic changes in the sperm that result in the release of lytic enzyme and exporure of part of the sperm structure known as the acrosoma reaction.As a result of these changes, the fertilizing sperm cell is able to reachd the doocyte, traverse its various layers and become incorporated

into the ooplasm of the egg:

CAUSES OF MALE INFERTILITY[11]

Causes may be:-

1.Pretesticular or mainly endocrinal.

2.Testicular or defective sperm production.

3.Post testicular or defective sperm delivery.

1. **Pretesticular causes** :-

Mainly constitutes endocrinal, other causes are stress. The various endocrinal defect leading to infertility are-

A. **Hypothalamic diseases**

* Isolated gonadotropin deficency (Kallman's syndrome)
* Isolated LH deficiency ("Fertileeunuch")
* Isolated FSH deficiency
* Congenital hypogonadotropic syndromes.

B. **Pituitary diseases** :

* Pituitary insufficency (Tumors,infiltrative processes, operation, radiation)
* Hyperprolactinemia
* Hemochromatoses-Approximetely 80% of these men have testicular dyfunction.
* Exogenous hormones (estrogen-androgen excess ,glucocorticid excess, hyper & hypothyrodism
* Hypothalamic diseases

Hypogonadotrophic state of male infertility

Depressed levels of gonadotrophins, in concert with subnormal level of testosterone & absent spermatogenesis, characterize the clinical state of hypogonadotrophic hypogonadism.

Causes of hypogonadotrophic state:-

1. idiopathic
2. Aquired

IN AQUIRED

A. **Defect in pituitary** :

* tumors of supporting structure
* Pituitary adenoma
* Anurysm of ICA
* An infiltrative process
* Radiation or operative

B. **Defect in hypothalamus**:

1. Primary & metastatic tumours
2. Infiltrative processess
3. Trauma
4. Infection.

In Idiopathic-

1. Isolated gonadotrophin deficiency (Kallman's syndrome)

* Familial disease having autosomal dominant transmission

* Generally presentation in adolescence with failure to progress through puberty[12]. However patients with partial deficency present in adult life with partial virilization with infertility & initial evaluation will reveal depressed LH, FSH & testosterone level.

2. *Congenital hypogonadotrophic hypogonadism syndromes* include Laurence-Moon-Biedl syndrome, the Prader-Villi syndrome & Moebius syndrome. All are associated with sexual infantilism secondary to hypogonadotrophic defects, but their presentation are not those of infertile males.

Two additional nutritional states resulting in hypogonadotrophic hypogonadism are malnutrition or severe illness. In these gonadotrophic level fall with concomitant decrease in serum testosterone & spermatogenesis. Prolonged starvation leads to testicular histologic changes. Alcoholism; in addition to its direct toxic effect on testicle; also leads to altered estrogen metabolism leading intially to depressed gonado trophin levels, followed by decreased testosterone levels & spermatogenesis.

HYPERPROLACTINEMIA

Indicators of pituitary-hypothalamic function that are reported to abnormal in hyperprolactemia are -

* Loss of periodicity of LH secretion[14].
* Loss of response to LRH[15]
* Abnormal secretion of LRH & normal response to LHRH.

Hyperprolactinemia has been demonstrated to have a direct effect on testicular function resulting in gonadal refractoriness to gonadotrophin stimulation[16].

Various studies have reveal, that there are two groups of male with hyperprolactinemia -

(1) Those with large tumors & high prolactin levels who

present with impotence.

(2) Those with nondetectable sites of hyperprolactin secretion who present with infertility. This latter group seems to be an extremely rare one.

THE THYROID

The thyroid gland exerts its influence on the testis in several ways -

a) Increases sensitivity of gonads to gonadotrophins.

b) Thyroid hormone can affect testicular metabolism[17].

c) Additionally, the thyroid has the ability to affect the rate of production of hypothalamic releasing factor & ant. pituitary hormones.

Alteration in thyroid function lead to decreased fertility Hyperthyroidism in man will lead to a constellation of manifestations of increased estrogen production, but the effect on fertility has not been investigated[18].

Hypothyroidism may lead to abnormal spermatogenesis with decreased total sperm count & decreased motility[19]. Generally these patients complain of loss of libido which usually is not accompoined by any alteration in sexual function[20]. It seems that hypothyroidism exerts its effect on spermatogenesis via reduced gonadotrophin release.

Thus, patients presenting for evaluation of infertility do not require investigations of their thyroid status unless there are overt clinical signs or symptoms of thyroid dysfunction. Subtle alternation in thyroid function seem not be related to fertility in man.

THE ADRENAL GLAND

The two pathologic adrenal status which have been implicated as potential cause of male infertility are-

1. Congenital adrenal hyperplasia
2. Cushing's syndrome.

1) Congenital adrenal hyperplasia-

Excessive adrenal androgens would lead to gonadotrophin suppression & thus infertility.

2) Cushing's syndrome.

* Five times more common in females than in males.

* Patient reported with Cushing's syndrome are found to have normal serum LH levels in face of decreased testosterone levels. This is due to decreased responsiveness of leydig cells due to high serum glucocorticoid levels in these patients of untreated Cushing's syndrome; on testcular biopsy can have following findings[21] -

i) Hypospermatogenesis (ii) Tubular thickening

iii) Disorganisation of the tubular epithelium.

These patients also have -

- loss of libido
- Impotence

Explained on the basis of reduced testosterone levels. Reversal of the impotence & imporved testicular biopsies have been documented with appropriate treatment of hypercorticoid state[22].

TESTICULAR CAUSES OF INFERTILITY

A. Chromosmal disorders

    1. Klinefelter's syndrome

    2. XYY syndrome

B. Vanishing testes syndrome

C. Noonan's syndrome

D. Varicocele

E. Myotonic dystrophy

F. Orchitis : Mumps and Leprosy

G. Cryptorchidism

H. Chemicals

I. Irradation

J. Ageing

K. Miscellaneous - Paraplegia, Polyglandular failure, Obesity, Sickle cell Anemia, chronic liver disease.

L. Idiopathic oligospermia

M. Germinal aplasia (Sertoli cell only syndrome)

N. Idiopathic testicular failure.

CHROMOSOMAL DISORDERS LEADING TO INFERTILITY

1. **Klinefelter's syndrome** -

Most common known cause of primary testicular failure associated with impairment of both spermatogenesis & leydig cell function.

Clinical presentation as small testes, azoospermia, gynecomastia, androgen deficiency & elevated urinary FSH. Not all patients share same findings.

These patients have an extra "X" chromosome in all their cell lines due to non-dysjunction of "X" chromosome during meiosis. Advanced maternal but not paternel age is associated with an increased incidence of these syndrome. Testicular biopsy in these patient shows-

-seminiferous tubular rarely contain germ cells.

- Sertoli cells mophologically abnormal.

- Leydig cells are prominent & hyperplastic.

Hormonal studies - FSH & LH

Testosterone - low normal or low

S. estradiol - Normal or increased.

XYY SYNDROME

- Usually have increased aggressiveness & criminal behavior[23,24].

- Usually tall stature.

- Rarely present with infertility due to impaired spermatogenesis[25].

Vanishing Testes syndrome (Prepubertal castrate syndrome; Anorchia)

Presentation as sexually innature males with no plapable testies due to testicular androgens.

Hormonal studies - FSH & LH with prepubetal level of testosterone.

Etiology :- exactly unclear but studies suggest -

testicular torsion, trauma or infection after fetal gonadal differentiation.

NOONANS SYNDROME

Clinical featurs : Short Stature, webbed neck, hypertelorism increased incidence of congenital heart desease, mild mental retardation, various skeletal abnormalities & underscended testes karotype : 46 + XY.

SCROTAL VARICOCELE

Amongst infertile males the incidence has been reported to be between 34-39%[26]. Actually, the scrotal varrcocele is the most common indentifiable & surgically correctable factor contributing to impaired testicular function & decreased seman quality.

Precise mechanism whereby a varicocele may lead to gonadal dysfunction remains unclear, although several theories have been proposed.

(A) Heat :- an elevation of only 2°C adversely affects quality of sperm production[27].

In human male, this heat effect has been cited as a contributing pathophysologic process in such entities as

cryptorchidism, febrile illnesses, prolonged & excessive use of hot tubs[28].

(B) Refluxing venous toxins : Macleod in 1965[29] proposed that an abnormal "chemical environment of the testes" may be a causative factor in depressed spermatogenesis associated with a varicocoele. Chemical environment may include -

"Adrenal metabolte or catecholamne metabolete through renal veln into internal spermatic vern"

(C) Pressure ischemia

(D) Blood stagnation with germinal epithelial hypoxia.

(E) Alternation in hypothalamic gonadal axis.

Size of varicocele does not appear to determine the magnitude of its effect.

ORCHITIS

MUMPS virus & acid fast bacillius of lepromatous leprosy may lead to testicular failure Rarely, uilateral suppurative infections of epididymis may extend to inclue the testis indequatecly or untreated genital infections with N.Gono cocuus may also occassionally cause orchitis.

Orchitis was observed in 30% of males who were 10 years of age or older when mumps parotitis occured[30]. Fortunately, most males develop parotitis before age of 10 years & orchitis is rarely observed in that age group. Bilateral orchitis develops in upto one third of the affected individuals resulting in subsequent presentation with severe oligospermia or azoospermia.

HISTOLOGY :- testicular atrophic changes

HORMONAL STUDIES :- Serum FSH & occassionally, LH may be elevated whereas testosterone levels are usually normal.

LEPROSY :- Testicular involvement occurs in upto 90% cases of lepromatious leprosy clinically, testicular atrophy - 10-20% of infected patients.

HORMONEL STUDIES :- FSH & LH & reduced testosterene level

MYOTONIC DYSTROPHY

- Autosomal dominant trait with variable peneterance.
- Upto 80% of affected males will eventually develop testicular atrophy.
- Besides involvement of muscles of distal extremities & cranium; other clinical features include prematre baldness. posterior subcapsular cataracts, cardiac conduction defects, impotence, rarely gynecomastia &, at later stages, variable degree of dementia.

CRYPOTORCHISM

Review of fertility in operated & nonoperated unilaterally crypotorchid patients has demonstrated a 62% fertility rate in patients operated on before puberty versus 46% in a nontreated group[31]. Eldrup & Steven have demonstrated extremely low sperm densities in ejaculates from testes that had been subjected to orchidopexy during childhood[32].

Hormonal studies : - Various conclusions have been documented, however as geminal epthelum becomes progressively damaged. the LH response & total plasma testosterone levels, however, were not significantly abnormal. This exaggereted FSH response may reflect abnormal feedback inhibition due to damaged sertoli calls.

CHEMICALS

Exogenous chemicals may affect the testis directly or indirectly Directly acting compounds : Flutamide, Cyproterone acetate, spiroinocaltone[33] & cimtidine[34].

- Various alkylating gents & antimetabolites.
- Nitrofurantoin impairs testicualr function in high doses inferfering with carbohydrate metabolism in germinal epithelium to produce a arrest at primary spermatcoyte stage.

Exposure to 1,2 dibromo 3 chloropropare in the pestcide industries has been observed to impair testicular function in man & melerfertelity[43] No chemical have been associated with effects as strong as DBCP.

Recent reports show that larger amounts of ethanol or its metabolites, actaldehyde, can directly impair synthesis of testosterone in man by increasing testosterone metabolic clearance rates & by suppresing gonadotropins[45].

Indirect acting compounds such as natural or synthetic androgeus, progestins & estrogens can supress the hypothalamic - pituitary axis to inhibit spermatogeneses. Estrogens may have a direct supressant effect on the testis[36].

An enoreased frequency of reduced speem count amang

workers expend to glycol ethers has be reported[45]. Resk of spontaneous obortion was increased among wives of men occupationally involved in orginic solvents handling in general or to have in particular[46].

IRRADIATION

Germs cells are particularly sesitive to radiation while the leydig cells are relatively resistant. The effect is dose dependent with no significant change in sperm density following exposure of the testis to either 8 or 20 rads but a 3 oospermia was observed in man who recieved more than 70 rads to the testis. Prepubertal testes seems to be more suscectable to radiation injury Longer periods are requried before spermatogenesis reappearis if the dose is increased to 100 rads. Spermatogonia are most rodiosensitive, spermatids & spermatocytes are relatively resistant, whereas leydig cells & sertoli cells are most resistant to irradiation.

MISCELLANEOUS

PARAPLEGIA :

Development of paraplegia after puberly has been associated with development of gyncomastia, impotence & testicular dysfunction with normal gonadotropin & subnormal testosterone. The exact mechanism implecated are unknown but probable are autonomic dysfunction & high scrotal temperature.

POLYGLANDULAR FAILURE :

The association of hypothyroidism & adrenal insuffciency with diabetes mellitus, hypoparathyroidism, pernicious anemia, vitiligo, alopccia & hypogonadism has been well established, Probable (mechanisms) are autoantibodies to various endocrcne tissues.

OBESITY :

Gross obesity may be an important factor causing male SUBFERTILITY. These patients have significantly reduced size & secondary sexual charactertics, conversion of T to E2[37] in the adipose tissue has been suggested the responsible mechanism. Semen analysis - oligospermia or impairment of viability & motility.

SICKLE CELL ANEMIA :

Males with sickle cell anemia frequently manifest impairment of statural and sexual maturation. Abbasi et. at.[38] have reported abnormal secondary sexual characteristics in 29 and enunchoide proportions in 31 of 32 patients. Testicular failure is ususally associated with elevated basal levels of gonadotropins and exaggerated responses with administration of LH-RH. Spermatogenesis has also been found to be impaired. Because zinc levels are frequently reduced in these patients and because zine deficiency and cause testicular atrophy in animals.

UREMIA :

Impotence dysfunction in the hypothalamic-pituitary testicular axis and impairment of spermatogenesis are common findings in uremic males[30]. In Men with CRF including those receiving chronic dialysis, impotence, oligospermia & germinal cell dysplasia are common, as reduced plasma testosterone levels. Like growth, sexual maturation is often impaired in adolescent children, even among those receiving chronic dialysis[47].

CHRONIC LIVER DISEASE :

In a review of 108 patients with chronic liver disease it was noted that importence was present in 79% gynecomastia in 52% and testicular atrophy in 47%.

POST- TESTICULAR CAUSES OF INFERTILITY

Account for upto 15%of cases of male infertility.Causes can be -

1. Disorders of sperm transport

(a) **Mechanical obstruction** : Account for upto 6 to 7% cases of infertility

1. **Congenital**: Can be due to-

'Atresia of the cauda epididymis or the proximal part of the vas deferens. It is amenable to surgical repair by epididymovasotomy.

Absence of the vas deferens may occur unilaterally or bilaterally.It may be accompanied by absence of the seminal vesicles or part of the epididymis.It is always associated with azoospermia, semen that does not coagulate at ejaculation, in absence of fructose.

Definitive diagnosis will require scrotal exploration.

Patients with cystic fibrosis also have a high incidence of congenital absence or hypoplasia of the efferent ducts and seminal vesicles.

Intrauterine drug exposure eg.diethylstilbestrol (DES)may result in obstructive epididymal lesions[40].

2. **Acquired**: Can be due to -

a. INFECTION: accounts for upto 40 to 50% cases of obstructive azoospermia.Gonorrhea was by far the most important bacterial agent,other agent can be E.coli, Pseudomonas aeruginosa, Staphylococcus aureus, and Streptococcus fecalis. Tuberculosis involving the epididymis and vas is usually diffuse and is secondary to prostatic or seminal vesicular infection. Bilateral infection is observed in 10 to 15% of all cases of genital tuberculosis. Treatment with chemotherapeutic agents may result in spontaneous recanalization.

b. TRAUMA: Apart from vasectomy for voluntary sterilization,vas may accidentally be ligated during hernia repair, orchiopexy, and even during varicocelectomy, hydrocelectomy and vasography.

Functional obstruction of sperm transport results from neuropathic insults like injuries to the sympathetic nerves during retroperitoneal lymph node dissection or pelvic surgery. This may cause lack of peristalsis of the vas deference with the resultant lack of emission and or failure of the bladder neck to close at the time of ejaculation leading to retrograde ejaculation.

DIABETIC males with autonomic neuropathy frequently present with both erectile dysfunction and or retrograde ejaculation.

SPINAL CORD INJURY can result in paraplegia or quadriplegia with resultant erectile dysfunction and lack of emission and ejaculation.

DISORDERS OF SPERM FUNCTION:Once the spermatozoa leave the male genital tract, surprisingly little is known about their subsequent behavior and physiologic function.The normal sperm function can result in impaired ability to penetrate the ova. Isolated sperm motility/viability problems may be congenital or acquired.

Factors associated with impairment of sperm Motility/viability

Congenital
- Kartagener's Syndrome[41]

Acquired
- Intrinsic-varicocele
  - Prolonged abstinence
  - Androgen difficiency
  - Epididymal dysfunction
  - Infection and occult disorders of accessory sex glands
  - Aggulutinating and immobilizing antibodies
- Extrinsic-drugs-Dilantin
  - Marijunana
  - Alcohol
  - Cigarette smoking[42]

SMOKING- Varioius detrimental effects of smoking on sperm concehtration ,sperm motility,and percentage of morphologically normal spermatozoa[48-52]. The effect of smoking on human Leydig cell function is controversial. Despite the reported adverse effects of smoking metabolites mcell. Inhalation of smoke, whether though active or passive smoking,, leads to absorption of these substances through the pulmonary vasculature and blood-borne circulation.

It is also that these substances couled end up in the seminal plasma of smokers via Various modes of diffusion and active transport.[49] Higher incidence of abnormally shaped sperm cells as well as decreased motility and sperm concentration in men who smoke.[53,54] Fluctuations in male hormones (androgens) and other hormones responsible for the regulation of spermatogenesis and sex drive have been documented in male smokers[49]. Seminal plasma obtained from smokers had detrimental effects on the sperm quality. SP from nonsmokers may contain a protective substance or factor involved in protection of spermatozoa against cigarette smoke metabolites and that this substance or factors may be decreased or inactivated in the SP of smokers[55].

Spermatozoa from smokers showed decreased sperm qualitative and quantitative characteristics.Although semen volume was not reduced in smokers, the spermatozoa count decreased by 26%. Spermatozoa from smokers exhibited lower motility and progressive motility characteristics. Lower motility and progressive motility problems have been associated with abnormalities noted within the ultrastructure of the flagellum and the axonemal structures of the sperm tail[56,57]. The most severe abnormality noted in the axoneme of spermatozoa from smokers was the complete disppearance of one or more of the nine fiber doublets and one or more of

the central fibers. Axonemal deficiencies are often the cause of lowered motility, progressive motility, and fertility in spermatozoa with a high incidence of defects such as those observed in asthenozoospermic specimens.

DRUGS-Drugs may impair androgen action through numerous distinct and somtimes multiple mechanisms-
Medications associated with testicular dysfunction and/or gynecomastia.

Anti androgens	**Antineoplastic drgus**
Spironolactone	Cyclophosphamide
Cyproterone acetate	Melphalan, Chlorambucil
Cimetidine	Nitrosoureas,Carmustine,
Estrogens &Hormones	Busulfan,Cisplatin,Cytarabine
Estrogen agonists	Procarbazine,Vinblastine
HCG hormone	
Anabolic steroids	**Psychoactive agent**
Growth hormone	Tricyclic antidepressants
	Amphetamines
Anti hypertensive /CVS agents	Narcotics
Digoxin[58]	Tranquilizers
Calcium channel blockers	**Others**:Phenytoin
Amiodarone	Ketoconazole [59]

Testicular Function in HIV-Infected Males: Testicular function is affected by the progession of patient 'sdisease as males clas-rifified as in class IV of disease have a reduced seminal output volume. These men have more frequently a reduced total sperm count, a reduced total motile sperm count, and a higher concentration of round cells.A reduction of seminal quality in males staged as in class IV of disease is supported by higher serum gonadotrophins levels recorded in

these males. The concentration of FSH is increased two-folds, the LH values are nearly tripled, and also Prolactin titres are higher in males with severe immunodeficiency, These data could be of help for counselling HIV-infected males willing to have a child[60].

Not all bacteria are considered pathogens however many Gram negative bacteria are more often thought to be pathogenic(Meares 1973). However, chronic nonsymptomatic prostatitis caused by gram negative bacteria have not been documented as a cause of male infertility (Amelar and Dubin, 1973). Whether Myoplasma and ureaplasma although frequently isolated, cause of infertility is not clear but Friberg and Gnarpe(1974) reported a conception rate of 24%following antibiotic therapy in such patients. Viral agents including cytomegalovirus and Herpes virus homins are found in semen of normal men(Lang and Kummar 1972). There have been no reports associating infertility with the presence of these viral agents.

I Mononucleosis and hepatitis can cause temporary but often marked depression in sperm production, wether these agents have specific effect on spermatogenesis is not known. Concern has also been raised on effect of severe fabrile reactions that accompany viral infections. Most of these effects however, are self limiting and normal sperm viability will be recovered within 12 months.

GENITAL TRACT TUBERCULOSIS: deserves special mention as tuberculosis is quite prevalent in our country. Tuberculosis of epididymis is generally a part of systemic tuberculosis.When genital tract tuberculosis alone is present either the prostate or seminal vesicle or both are involved in 100% of cases while epididymis in 62% of cases. Most cases develop gradually with little pain. Tuberculous orchitis as a primary

infection is rare but tubercular epididymitis will eventually involve the testes and cause cessation of spermatogenesis at later stage of disease.

Lastly among the causes of infertility is factor which has of all factors received attention very lately and these are immunological factors.

IMMUNOLOGICAL FACTORS IN MALE INFERTILITY:

It is well established that antisperm antibodies (ASA) are etiologically implicated in female infertility. In general, the presence of ASA will reduce the likely occurrence of a pregnancy. The presence of ASA in the female reporductive tract may impair sperm-egg interaction by interfering with the dispersion by interfering with the dispersion of cumulus mass and sperm binding, penetration of the sperm in to the zona pellucida, and sperm egg fusion.

The presence of ASA in the male reported ductive tract affects affects sperm function by possbly causing premature acrosome reaction and sperm immobilization (agglutination) as well as by decresing membrance intergrity and opsonizing sperm for phagocytosis. Antisperm antibodies may bind to the sperm surface within the testis or epidiymis (before ejaculation) or during the mixing of the sperm and seminal plasma at ejaculation[63]. Both male and female can be rendered infertile by immunization with sperm[61]. Sperms are very antigenic and normally isolated from body, disruption in this anatomic and functional barrier in seminiferous tubules can lead to antibody formation. Sperm autoimmunity is the most common medically treatable condition seen in men with infertility. It is characterised by immunoglobulins coating the sperm, immunoglobulins localized in the intestitial spaces/tubular wall/ both, the presence of sperm antibodies in semen and blood of both the male and female partners and a variable semen quality ranging from azoospermic to normal[62]. Sperm antibodies can be present in fertile and infertile men at low levels insufficient to further impair fertility.There are two groups of

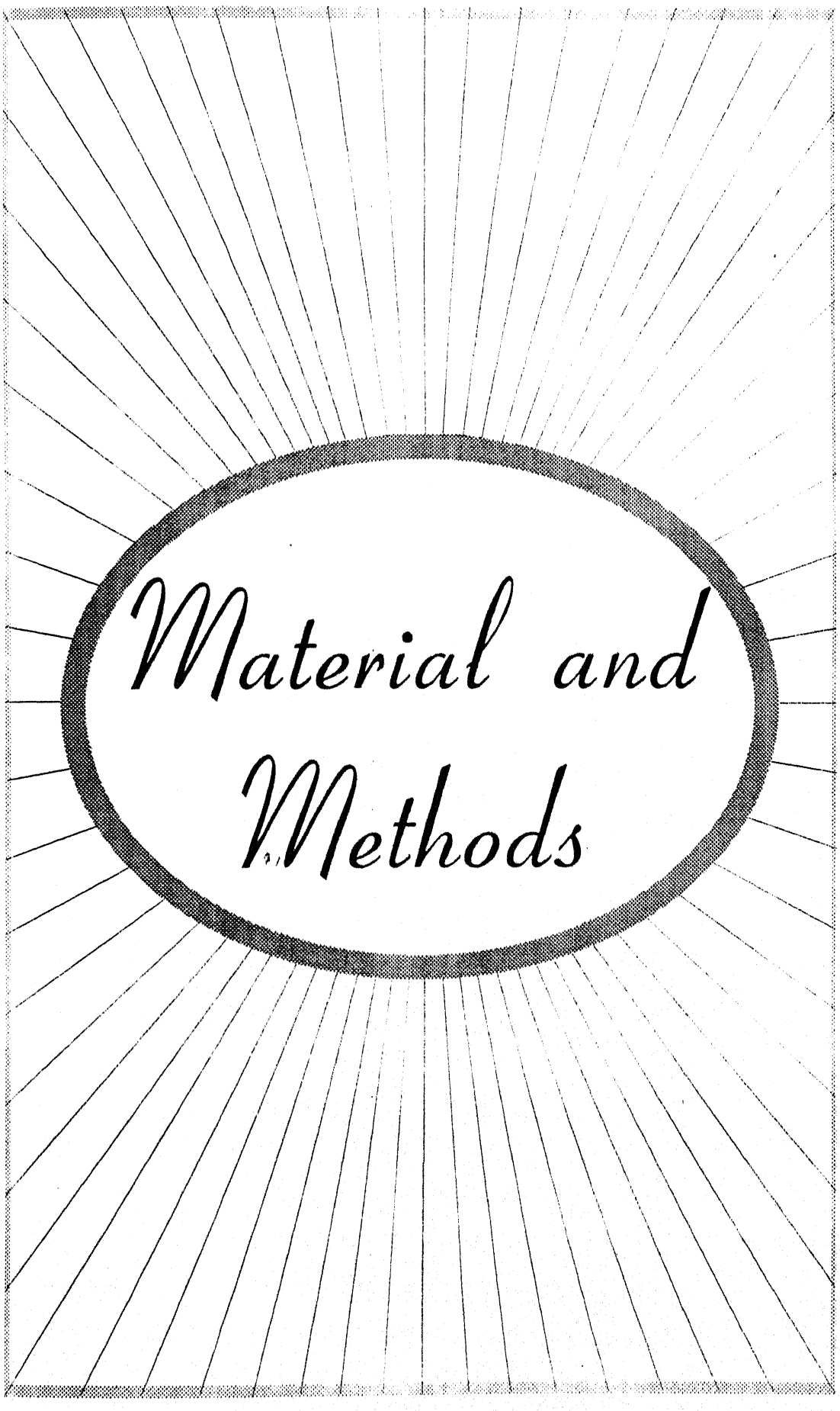
patients with anti sperm antibodies. One is in whom antisperm antibodies are associated with andrological problem causing disruption in blood-testis barrier as mentioned above i.e. (prostato-vesicular inflammation,orchiepididymitis, testicular injury, torsion of spermatic cord, ligation of spermatic cord[63]. In this group antisperm antibodies not neccessarily cause infertility. Second group is made up of subjects, whose antisperm immune reaction is the only evident factor and which could play a pathogenic role in infertility.

Antisperm antibodies have been found to be present in 17 to 30% of men in various reports.

In the worldwide survey W.H.O found immunological factor be suspected as cause of infertility in 2.8% of males consulting for infertility.In another report fertile men with history of vasovasotomy, 1 to 2% may be expected to have sperm bound antibodies, but the percentage is 7-14%.In men attending a fertility clinic and in 70% of them, there appears to be no known cause.

PSYCHOSOCIAL STRESS AS A CAUSE OF INFERTILITY:

Psychosocial stress plays an important part in etiology of some forms of infertility.Emotional factors contribute to about 25% of all infertility.One marriage in 10 is involuntarily infertile. The sexual and psychological problems of the infertile couple, however, have been frequently overlooked or knowingly neglected.Anxiety must be reduced to the point that the patient can talk about sexual performance and dysfunction.Stress may significantly alter both spermatogenesis and ovulation to affect fertility in men. The stress must be extreme in nature,however; the effects of daily stress or environmental contamination and nutrition on infertility is not adequately known but it is certainly reasonable to assume that these factors would play an important part in infertility.


Material and
Methods

MATERIAL AND METHODS

The present study was conducted in the department of Medicine M.L.B. Medical College, Jhansi. Patient were selected from the patients coming to the out patient room of the Reporductive Medicine clinic of the Department of Medicine of this college.

Male partners of 78 couples complaining of infertility was selected for this study. The infertility was defined as "unprotected barren union for 1 year during which the husband and wife should have stayed together."

Investigation of the Male partner begun with history, the patients History was noted down in the following format.

HISTORY

Name : Date of appointment:

Occupation : File Number :

Religion : Address :

Age and date of birth :

Wife's name :

Wife's age:

Socioeconomic status :

How long have you been married ?

Are you using/ used any contraceptive method (specify)?

How long you have been trying to have a baby?

Have you been married before?

Has your wife been married before?

Have either of you achieved a conception either with present partner or with another partner? Yes No

If Yes	Present Partner	Another partner
Husband	---	
Wife		

Are you currently taking any form of medication ? No Yes

(If yes please specify:

Family history

How many brothers do you have ? Number None

How many sister do you have ? Number None

Are you aware of any member of your family including your parents having had any difficulty in starting family ?

(If you are, please specify :

History of any of the follwing disease

Asthma or other allergy

(If other please specify :

Bronchitis, Orchitis, epididymitis, Prostatitis, Gonorrhoea, Urethritis, Varicocele, Hydrocele, undescended testes, injury to testes, urogenital tuberculosis, Pneumonia

Any other disease of the chest

Diabetes mellitus

High blood pressure

Frequent headaches

Mumps

(If yes how old were you when you had it and do you remember if the disease affected your testicles?

Hernia :

(If yes please say where it occured :

Chicken pox : Varicose veins :

Haemorrhoids : Back pain :

Any other disease not listed above : H/O Radiation :

Have you ever had a major surgical operation?

Have you ever had an operation of any sort associated with your penis or testicles?

If yes please specify :

Have you had any conditon in the past two to three years which caused you to have a fever or a significant increase in body temperature (e.g. flu)?

Do you suffer from recurrent pain in the testicles?

(If yes please indicate the frequency of occurence and state whether the pain is an ache or a sharp pain :

Do you feel a burning sensation in your penis when you urinate?

What on average is your frequency of ejaculation ?

Are you aware of any difficulties at intercourse on either your part or that of your wife or partner?

Indicate your weekly intake of alcohol.

Do you smoke?

If yes specify what, and if cigarettes specify the number per day & duration

In your daily work are you regularly exposed to heat or chemicals?

Developmental History And Exposures :

Developmental history is critical as this can indicate problems with formation of the genitalia that may suggest androgen deficiency or resistance which can present with hypospadias. bifid scrotom, or more marked forms of ambiguos genitalia. The parents should be questioned regarding the nature of his mothers pregnancy, duration, complications, and whether any medication were taken. In utero exposure to diethylstilbestrol (DES)· ean cause epididymal cyst and and an increased incidence of cryptorchism, Late descent of the testes as an infant, or incomplete descent, can indicate a

partially cryptorchid state with risk for impaired spermatogenesis as an adult.

Sexual history :

The sexual history provides an important information regarding erectile function, frequency of intercourse, and sexual techniques. Erectile dysfunction is usally the first clue to hypogonadism in the adult male. Onset and duration of impotency and presence or absence of noctural or morning erection with a full bladder can help to evaluate for psychogenic versus organic cause. Loss of morning erections correlates well with an organic cause. Sexual techniques can provide clues for both evaluation of erectile dysfunction and also for infertility in cases in which coitus may be poorly timed or fail to provide deposition of sperm in the vagina. With the infertile male, a history of previous pregnancies can suggets an acute change rather than chronic process and increase the likehood of a reversible condition. Also,the partners history should be determined to confirm that she has received adequate evaluation as a potential cause for infertility of the couple.

And lastly patients should be askeed for history of urinary symptoms. These patients have a higher prevalence of azoospermia and abnormal semen quality, particularly abnormal sperm morphology. As many as 27% men with such a history present abnormalities in expressed prostatic fluid and semen, suggestive of chronic male accessory gland infection. A history of urinary symptoms is also more common in men with varicocele.

PHYSICAL EXAMINATION

Physical examination included general examination and genital examination & systemic examination.

GENITAL EXAMINATON was noted down by filling out the following form for each patient in a manner. Already mentioned on detail in the review of literatutre.

(1) Penis	Normal Size.	Subnormal size
	Foreskin - Retractable	(Yes /No)
(2) Testes	absent	subnormal size
	Normal size	rudimentary
Consistency	Firm	Firm to soft
	Soft	not accessible

Other changes :

(undescended testes, fixed, hydrocele, varicocele, cicatricial changes)

(3) Epididymis	Normal	Hardened
	Thickened	Enlarged

Any othe pathological findings

(4) Prostate

(5) Secondary sexual characteristics

Examination of the patient should be made with the doctor sitting and the patient standing before him.

TECHNIQUES

The penis :

First note the size of the penis and find out if a phimosis is present, by drawing back the prepuce. If there are complaints about the erection,search for hard spots or ridges (induratio penis plastica). The is not uncommon to find presence of a hypospadias.

SCROTUM

Abnormalities of the scrotum concerns the shape,the skin or the contents. Especially in obese men the scrotum may be wider above than below,of the infantile type. On the other hand, some patients may have a very long scrotum. Both these anomalies in shape are often seen together with subfertility.The scrotal skin should be examined for the presence of psoriasis,eczema, lymphangioma.

VASA DEFERENTIA

Let the contents of the scrotum above the right testis roll between thumb and second finger of the left hand. After some practice, it is easy to distinguish between the rather soft veins and arteries and the much harder cord that is the ductus deferens. Follow the ductus deferens from as high as possible to the cauda epididymis and search for irregularities, hard spots or interruptions. Thereafter repeat this for the left side using the right hand.

EPIDIDYMIDES

Palpate the epididymis from the head to the tail, note if the epididymis is lying closely to or distant from the testis.This is especially of importance if the testis is small. If there is room between the testis and the epididymis, it is probable that the testis has atrophied, otherwise a congenital hypooplasia is more probable. Search for irregularities in the consistency of the epididymis. Small cysts are often found above the testis, without clinical significance. Sometimes a number of small cysts are felt in the head of the epididymis and this is of more importance. It may be a cystic degeneration with obstruction at several points. These

cysts may also give rise to the production of antibodies (Hamerlynck,1970).

TESTES

The position of the testes in the scrotum has to be stated. Normally they are lying immobile in the scrotal sac. In obese men they have the tendency to retract into the subcutaneous tissue or even into the external inguinal ring. This may be a cause of disturbance in spermatogenesis.

The testes have to be measured, which can be done in two different ways. One method is, to measure first the longest diameter of the testis with the aid of caliper. This will add to the measurement only a small error, which can be neglected, then one measure of the width, which is somewhat less reliable. One has to note a largest and a smaller width, because the girth of a testis is not a is note a circle. from these three measurements the voume of the testis can be computed fairly accurately. Complicated formula have been presented for this purpose. less accurate but more suitable for practical use is the relation between the largest diameter (the length) and the volume of the testis as worked out by Hynie. the mean volume of a testis lies between 20 and 30 ml.

The accuracy of measuring the diameter of a testis depends on the consistency. If the testis is rather soft, it is arbitrary how much pressure should be applied with the calipers. This can make quite a difference, especially for the largest diameter. This disavantage can be overcome and even made to yield profit by taking two 'extreme', measurement. One with as much pressure on lthe calipers as can be applied without hurting the patient; this is the minimal longitudinal dimension. Thereafter the testis is compressed. firmly on the transverse diameter with thumb and fingers of

the other hand and now a second measurement of the length is taken without any pressure of the calipers; this is the maximal longitudinal dimension. The difference between the minimal and the maximal longitudinal dimensions normally is less than 10 mm in testis of normal size. If the consistency is too soft, the diffrence is 10 mm or more.

For the largest diameter the normal figures range from 40 to 50 mm. The mean width of a normal testis lies between 20 and 30 mm and the error of measurement will only be small. Thus the figures for a normal testis will read : 51/43 X 26, meaning that the maximal logitudinal dimension is 51 mm, the shortest 43 thus the consistency is normal as the difference is less than 10mm, The mean width is 26mm, For a testis of normal size but soft consistency this will read 54/40 X 25.Too small a testis will show a figure as 39/32 X20.

ABNORMAL CONTENTS OF THE SCROTUM :

Apart from the presence of a hydrocele or a spermatocele, it is of great importance to examine for the presence of a varicocele. This is rarely found on the right side, sometimes on both sides but most often on the left side only. The examination should always be done with the patient standing upright. If the patient is lying down, the varicocele may disappear completely. Let the contents of the scrotum above the testis roll through the fingers, then ask the patient to press by blowing on the back of his hand. The varicocele is noted as small (not visible from the outside,) It is most important to note whether the varicocele gets bigger in pressing because this indicates that reflux occurs. One should try to describe the degree of increase as acurately as possible, for instance : from small to moderate; or small increase in size

but not to moderate.

PROSTATE :

The next stage is the rectal examination, this can be done either with the patient lying on his back, or standing and bending forwards. The size and consistency of the prostate should be noted especially whether there are irregularties, hard knots, or other signs of past or present inflammation. This rectal examination and pressure on the prostate gives an embarrassing sensation to the patient and it is recommended to put a generous amount of oil, or preferably some ointment on the glove,a dry glove will make this examination unnecessarily painful. If a prostatitis is suspected, one has to ask the patient whether the sensation is merely disagreeable or really sharply painful.

Normally the seminal vesicles cannot be palpated rectally, except by examiners with unusually long fingers. One should not confuse the side lobes of a butterfly shaped prostate with the seminal vesicles. If one or both of these glands can be felt easily behind the prostate, there is definitely something wrong.

SECONDARY SEX CHARACTERISTICS:

After having completed this examintion of the patient's genital organs, one should give some attention to the secondary sexual characteristics. Of the secondary hair it is noted whether the upper limit of the pubes is horizontal, or of the male type. Is there hair on the breast? Is the beard fully developed, or are there smooth zones on the cheecks? Furthermore,

one should never forget to look for the presence of gynecomastia. It may also be of importance to note the voice and the mannerisms of the patient.

INVESTIGATIONS:

First part of investigations included routine investigations done in the following manner

	Examination	In what Patients
Blood	HB TLC DLC, ESR	All
	VDRL. B. sugar	All
	B. urea	As needed
	S. creatinine	As needed
Radiological	chest X ray	
	X ray skull	As needed
	USG Abdomen	As needed

SEMEN EXAMINATION was generally based on recommendations of the WHO laboratory manual for the Examination of human seman semen and semen cervical nucleus interaction.

INSTRUCTIONS TO THE PATIENT

The semen should be brought for examination at least twice, first after period of abstinence of 3 to 5 days, to have a standard for comparison ; the second time after a period of continence that is normal for the patient. If a couple has intercourse everyday,it may well be that a specimen produced after 4 days of abstinence is quite normal, but with daily intercourse the sperm count, as well as the volume may be too low. The investigator could miss

his important factor if he sticks rigourously to an examination after a period of abstinence of 4 days.

There are several ways for the patient to produce his semen for investigation except that an orgasm is always necessary. Fluid expressed from the penis by rectal massage, or obtained by puncture of the testes or epididymis, is not representative for the ejaculate produced by an orgasm. Also unacceptable is the examination of the drop of fluid that can be expressed form the penis after intercourse. Examination of the reflux semen after nomal coitus used to be the method of choice for roman catholic patients when the religious doctrine was more severe than in later years. This method has the disadvantage that the collection often is incomplete, that the biochemical constitution may be greatly changed and that leucocytes and epithelial cells from the vigina may interfere with the judgement. Much better is the method of coitus interruptus, although this has the disadvantages that the very first part of ejaculate may be lost, if the patient is not quick enough. Total recollection is guaranteed by using a condom, but his is to be depreciated because many condoms or the powder therein will inferfere with the motility of the spermatozoa (pseudonecrozoospermia). Ignorance of this possibility has been the cause of the many misapprehensions in the past. If massage is not acceptable on religious considerations, the cervical spoon may be used (Doyle 1948 and Schellen,1958) although in this way one can not be sure that the complete ejacualte is recollected.

The best method to obtain the semen for investigation is the production of an orgasm by means of massage of the penis, either by the patient or by his wife. For this procedure it is better to dispense with the 'loaded'word masturbation. Theree is no difference between the seminal patterns if the semen is produced by massage or by interrupted coitus, as has been proved by various seminologist (Freund, 1962).

The semen has to be collected in glass jar or a plastic jar, supplied by the andrologist. This jar must have been tested and found harmless to the motility of the spermatozoa. The receptacle should be kept at body temperature for some time before usage, to prevent cold shock. The semen has to be transported at out-of door temperature. Only if this is near freezing point should it be kept under the clothes during transport. The specimen has to be delivered within two hours after ejaculation, preferably within one half hour.

COLLECTION

Two semen samples were collected from each patient. Sample was collected after minimum of 48 hours and not longer than 7 days of sexual abstinence and the two sampls were collected not less than 7 days and not more than 3 months apart. If semen showed any abnormality, an attempt was made to collect a third sample but this time without abstinence at usul ejaculatory to low in examination after abstinence might in usual ejaculatory frequency become subfertile or infertile. This is because it has been shown that

when frequency of ejaculation is high the sperm counts and morphology is lower that what is after abstinence. So a person who is fertile on examination after abstinence might in practice be at borderline levels of fertility Semen was collected in a wide mouthed glass jar with cork by masturbation at the premises of Hospital only.

After each collection patient was asked to provide following ingormation. This was important. This information is important for the person analyzing the semen and helps in interpretation of the results.

1. Time of collection
2. Time of analysis
3. Was any semen lost at this collection Yes No
4. Was the semen thick
5. When did you last ejaculate
6. With this masturbation did feel you
   (a) Produced more semen than at intercourse
   (b) Produced less semen than at intercourse
   (c) Produced the same quantity as at intercourse
7. In the past 2-3 months did you have
   (a) had any major illness
   (b) had unusual alcohol consumption
   (c) taken drugs (specify)
   (d) Recent trauma to testes
   (e) had periods of constant stress
8. could your semen be infected (hepatitis B, HIV, sexually transmitted disease)-specify

Analysis The specimen was analyzed within 3 hrs. of collecton. The volume was measured to the nearest volume in ml. Before further analysis the semen was vigorously shaken in the container. This was because semen contains fluids from various organs with differing viscosity, cellularity in various portions. Also with time the motile cells tend to settle as a function of time.

A. THE SEMINAL PLASMA

1. VOLUME

The volume of an ejaculate depends mainly on the contribution of the seminal vesicles. Normally this lies between 1.5 and 5.0 ml. A smaller part is derived from the prostate, 0.2 -1.0 ml and from the glands of Cooper and Littre, a few droplets only.

About 0.2 ml, containing the spermatozoa, is derived from the vasa deferentia and the epididymides.

The average volume of a normal ejacualate after a period of abstinence of about three days. lies between 2 and 4 ml. The volume is farly constant in most patients; the upper limit is reached after a abstinence of aboutl four days. A volume of less than 1.5 ml or more than 6-8 ml is considered as abnormal.

PATHOLOGY :

Low Volume : If the volume of an ejaculate is less than 1.5 ml there are several possible causes.

1. In complete collection of the ejaculate. Repeated examination after re-instruction of the patient is necessary to exclude this factor.

2. Too short a period of continence. After an ejaculation, the seminal

vesicles need a time to be fully repleted. In most men the volume of the ejaculate is again at its upper limit after about three to four days of abstinence.

3. The manner of producing the orgasm. The manner of producing the orgasm for the collection of the semen does not have much influence. When masturbation specimens are compared with ejaculates produced by interrupted coitus, the difference is negligible with sporadic exceptions. However, if the ejaculate is produced by a normal coitus, whereafter the wife expresses the semen (reflux method), part of the ejaculate may well be retained in the vagina.

4. Incomplete orgasm. Psychological inhibition may cause a low volume as well as other abnormalities in the semen. One has to ask the patient for this possibility, and repeat the examination. The best way to get information about the orgasm is to ask the patient how many pulsations he notices. In some men the orgasm is of very short duration with only one or two pulsations and this may well be the cause of a low volume. If the patient notices four pulsations or more, and there is nevertheless a low voume a disturbance in the function of the seminal vesicles is more probable.

The disturbance may range from a small ejaculation with only a few droplets of semen plasma, to a slight inhibition of the contractions with much influence on the volume.

5. Abnormal function of the seminal vesicles. This may be caused by a congenital anomaly, possible in different degrees.

(A) High volume: A volume of more than 6ml usually points to an overdevelopment of the seminal vesicles.

One has to make sure that there is only seminal plasma and no urine in the ejaculate. Some patients have a disturbance in the normal reflxes during orgasm, causing admixture with urine.

Relation to fertillity

Low volume : Although usually a volume of less then 1.5ml is considered as abnormal, this does not necessarily mean that the fertility is decreased. The importance of a low volume by itself and as a single factor is often exaggerated. It has often been presumed that a low volume prevents the formation of a vaginal pool,or that a small amount of semen is incapable of buffering the acid vaginal secretion. In this respect, other factors also play a part; the position of the cervix to the vaginal pool,the acidity of the vagina and the occurrence or absence of orgasm in the wife, amongest others, However, a small volume may point to an abnormal function of the seminal vesicles and in that case there will indeed be a decrease in the fertilizing capacity because there are more factors involed.

High volume : It is often said that too high a volume (polyspermia) may have an adverse effect on the fertilizing capacity, because the sperm density will easily become too low, This is contrary to the situation in cattle, where the fecundity is requesterted by diluting the semen. Be this as it may,one should realize that the greatest number of spermatozoon is to be found in the first 1 or 1.5 ml of the ejaculate. This first fraction is requested against the eternal

orifice of the uterine cervix, thereby reaching the mucus plug. What happens with the rest of the ejaculate is, most probably, of far less importance. The vagina of a woman who has never given birth contain more than 1.5 ml semen. One can easily make the experiment by injecting into the posterior fornix a fluid of approximately the viscosity of semen. The higher the viscosity, the more will be retained, but rarely more than 2 ml. Consequently, all of the ejaculate above this limit will be spilled as the so-called reflux. It does not seem to be of much importance whether this reflux measures 1 or 4 or even 8 ml. as long as the first part of the ejaculate reached the cervical mucus first.

PH:

The initial PH depends mainly on the relation between the alkaline secretion of the seminal vesicles and the acid secretion of the prostate. After ejaculation the PH tends to decrease due to the formation of lactic acid from glucose and fructose, especially if the motility is good.

The normal PH ranges from 7.4 to 8.5 if measured within one hour after ejaculation.

PATHOLOGY :

(a) Low pH. A low pH points to a disturbance in the relation between the secretion of the seminal vesicles and the prostate, usually because of a deficiency of the former. Consequently a low pH is often seen in semen specimen with a low volume. The extreme example is the patient with congenital absence of the wolffian ducts, a pH below 7.0

can be considered as a confirmation (Kremer, 1967).

(b) High pH. A pH above 8.6 is considered as abonomal, although there is no valid reason for this, according to some authors.

RELATION TO FERTILITY

An abnormally low pH,although in itself no reason for a decrease in fertilizing capacity, is an important finding because of the usually accompanying abnormalities (low volume, low content of sugar).

VISCOSITY

The viscosity of the semen is the degree of stickiness that remains after liqiefaction.

PATHOLOGY

Low viscosity. Semen with a low viscosity tends to spread in the vagina. This dilution along the walls may interfere with the formation of a vaginal pool and thus lessen the fertilizing capacity of the semen. This is a theoretical conception that has not been proven.

High viscosity. High viscosity may cause a decrease in the fertilizing capacity because the spermatozoa may not be able to pass from the semen into the cervical mucus.

FRUCTOSE AND GLUCOSE

Fructose. Mann published in 1945 that in animals the reducing substance in the semen, hitherto held to be glucose, was mainly fructose. This was rapidly confirmed for human semen. The fructose in the semen is exclusively produced by the seminal vesicles.

In normal males, there is a wide variation in the

fructose content of semen from 6 to 30 mmol/l. The content of fructose is mainly dependent on the production of testosterone. At first a close relationship between the activity of the cells of Leydig and the content of fructose was postulated (Nowakowski and Schirren,1956). However, seminal fructose is now considered as an unreliable parameter for androgenicity.

It is not clearly understood why seminal fructose diminishes greatly after sexual continence of more than two weeks (Schirren, 1963).

In semen with a normal number of motile spermatozoa the fructose decreases gradually to about half the initial value after four to five hours. This process of fructolysis is not present in azoospermia nor in necrozoospermia. Some andrologists lay a great value on the estimation of the fructolysis as a parameter for normality of the spermatozoa (Peterson and Freund, 1971).

Glucose - The normal values for glucose lie between 0.01 and 0.2 μmol/l.

PATHOLOGY :

Low fructose. Too low a content of fructose in the semen is not at all rare in patients who visit the fertility clinic because of a childless marriage. This is encountered less frequently in men of proven fertility. The cause is always to be found in the seminal vesicles. In most patients the lack of fructose is irreversible and resistant to treatment with androgens. The cause is most oftenly can acquired (post inflammatory) deficient function of the vesicles or sometimes

a congenital anomaly.

Low glucose. Glucose is absent in congenital absence of the Wolffian ducts. It is sometimes absent in otnerwise normal semen. The backgraound of these cases has not been studied as yet.

High fructose. If a a patient has abnormally high content of fructose in the semen, it may be that the prostatic fluid is lacking and that the production of fluid by the seminal vesicles is low.

Glucose. In the laboratory of the Department of Obstetrics and Gynecology of the Vrije University at Amsterdam (Head Dr. H. van Kessel) glucose is not increased in every patient with diabetics. Sometimes a high content of glucose is found (4.0 mmol/L or more) without any explanation.

RELATION TO FERTILITY.

Low fructose. It is often stated that a lack of fructose in the semen is accompanied by low fertility.

High Fructose
Low glucose
High glucose

no data available.

PROSTATIC ACID PHOSPHATASE

The content of the seminal plasma should be above 200X10 μ/l.

PATHOLOGY :

In patients with gross abnormalities of the seminal vesicles, as in the congenital absence of the Wollfian ducts, the content of prostatic acid phosphatase lies above one

million μ/l (Hellinga et al.1971). In normal patients such high figures are sometimes found with normal function of the seminal vesicles; no case for this anomaly is known.

A low content is often found in combination with low motility and this is presumed to be caused by a diminished function of the prostate.

RELATION TO FERTILITY

There is no sound arguement that low ontent of this compound goes with low fertility.

PROSTAGLANDINS :

Several prostaglandins are present in the seminal plasma. The seminal vesicles are the source of origin (Eliasson,1969).

Pathology

Already in 1947 it was found, other still by bioassay, that semen from men has a lower prostaglandin concentration than semen of proven fertility. This seems to depend especialy on the concentration of prostaglandin E compounds.

Relation to fertility

From the investigations of Bygdeman et al. (1970) the conclusion seems to be warranted that E compounds have some relationship with fertility. The nature of this relationship and whether prostaglandin E is in itself of importance for the reproductive processes, is still uncertain.

THE SPERMATOZOA

SPERM COUNT

Formerly it was presumed that a density of less than 60 millions per ml were too low but now put lat 20 millions per

per ml. This was first stated by Mcleod and Gold (1951) who made a follow up study on two groups of one thousand males. The first group had recently impregnated their wives, the second group is thought aid for a childless marriage. They found in both groups that with a density above the 20 million level a further increase in sperm count did not increase the case of conception.

In most males, fertile or infertile, the sperm count varies within rather narrow limits (Mcleod and Gold, 1951). There are exception however,and in some patients the fluctuations may even be up to five or tenfold.

Pathology

Low density

Oligozoospermia may be an artifact. If the first part of an ejacualte is lost during collection, the density in the collected second part will be low. Also an incomplete orgasm caused by psychological inhibition or a cramped state of the smooth muscle tissue may have the same result. Careful interrogation and reinstruction of the patient will reveal this possible factor.Furthermore, too frequent ejaculations and too short a period of continence will result in oligozoospermia in the most men.

Rarely a low density is the result of too high a volume. Then, oligozoospermia without other abnormalities in the semen may be caused by too low a number of normally functioning tubules. It may be that the testes are too small or that part of the tubules are fibrotic, caused by some

severe illness or inanetion in the past. In still other cases, a situation develops where the germinal tissue dissappears from the tubules, only the cells of Sertoli remaining. If fully developed this syndrome of depopulation of Sertoli cell only syndrome causes azoospermia. In the incomplete form of partial aspermatogenesis, there will be oligozoospermia.

Hypospermatogenesis that is a diminished activity of the spermatogenec tissue, usually leads to oligasthenozoospermia. If there is disorganisation in the tubules oligo-astheno-teratozoospermia will be the result.

Polyzoospermia

A density of more than 250 millions per ml is considered as abnormal. Doepfmer (1962) made an analysis of cases with a high sperm count and he distinguished different situations. If a high density is caused by a relatively low volume in the presence of a normal total count, this is relative (pseudo) polyzoospermia. Absolute (real) and the total sperm count more than 600 millions. This is caused by overactivity of the seminiferous tissue, with normal function of the accessory glands. In these patients there is a relatively high percentage of cases with head to head agglutination in the semen and sperm agglutinins in the blood.

Relation to fertility

Oligozoospermia

A sperm count of less than 200 mellions per ml is the cause of a low fertility, even if the total count should be within normal limits (Mcleod and Gold (1953). In the human,

only a few ml. semen usually only the first sperm rich part of the ejaculate, will come into contact with the cervical mucus. A great deal of the ejaculate, depending on the total volume, will flow back out of the vagina. It is not important how many spermatozoa are lost in the reflux semen. Therefore it is the density of the sperm cells, especially in the first ml, that is decisive. In most semen samples with low density, the subfertility is increased by the presence of other factors, an accompanying low motillity or high percentage of morphologically abnormal sperm cells or both.

Technique:

Sperm count was done by diluting the semen to 1:20 with semen diluting fluid in a WBC pipette and counted on Neuber's hemocytometer after the sperm are allowed to settle and fixed in 10% formalin.

If no spematozoa are found in motility examinations were made at x 150 and x 600 on 5-10 microscopic fields in each of 2 wet smears. If no sperms are visualized in 50-100 low power fields the semen was centrifuged for 10 minutes and sediment centrifuged and again visualized before declaring the semen azoospermic.

Polyzoospermia

In relative (pseudo) polyzoospermia subfertility is the rule. This is caused by the deficiency of normal plasma constituents in these cases. Real polyzoospermia does not decrease the chances for pregnancy, although there are indications that in

many instances the pregnancy will end in an abortion.

MOTILITY

As long as the tightly packed spermatozoa are in the epididymis and in the ampulla of the ductus deferens, they remain immobile. As soon as they are expelled by ejaculation, entering the secretions from the prostate and the seminal vesicles, they respond to the change in environment with an outburst of motility. With regard to the motirity three different factors are distingusihed. The qualitative motility gives the percentage of spermatozoa that are in any way not immobile. The qualitative factor gives the degree of motility of which at least four grades have to be distingusihed (1) progressive forward propulsion; (2) weak forward movement; (3) movement on the spot without displacement; (4) Immobility, As a third factor more and more importanceis has ganed to reguarding the type of motility. (a) in the normal type of motility the tail beat, generated from the midipiece and also for a small part from the tail structure. (b) If the tail is a weak forward movement without rotation. (c) Swiming in circular orbits in one plane only (yawning spermatozoa) is probably caused by a restricted activity of fibrillar system in the tail (Van duijn et al. 1966)[64].

Normal figures for the motility are that ther should be at least 40 percent of the sperm cells either normal rotation forward propulsion (grade 1a) withing two hours after ejaculation.

In many patents the motility in the first part of a split ejaculate is better than in the complete semen (Eliasson,1972)

Technique: Two seperate drops of raw semen are placed on a glass slide and the slide was placed in a incubator at 37 degree for 10 minutes.

This is because at room temperature motility has been shown to be greater than at lower temperature 20° C. Also examining motility at body temperature is optimal. This has been recommended by various authors (Fruend 1974, Janick and Macleod.

5-10 microscopic fields were examined (at x150 and x 600) in each of these 2 wet smears percentage motility was rated.

Motility was rated as follows % with

- Linear forward progression
- Slowly progressive
- Non progressive
- Non motile

Motility was rated as % of motile sperms

Pathology :

A low motility of the spermatozoa is called as asthenzoospermia. This may be quantitative, with too low a percentage of motile sperms. Or it may by q ualitive, if the degree of motility is insufficient. Abnormal types of motility are named as such (stiff tail beat; yawning sperms). Decreased motility may have its cause either in the spermatozoa themselvs or in the contents of the seminal

plasma theoritically the possibilities are as follows.

1. Asthenozoospermia without any other noticable abnormality in the seminal pattern, in 9 out of 10 cases is an artifact. This is true as will for qualitative as for quantitative deficiences. The cause may be a faulty technique: too quick cooling (cold shock), use of an unclean glass jar; moisture; mixture; mixture with acid vaginal secretion; use of a preservative or condom. Repeated examination after reinstruction of the patient is necessary in all cases of asthenozoospermia.
2. If the asthenozoospermia is caused by a deficient function of the seminal vesicles, this may be accompanied bya low volume and a low content of fructose. Also the acid phosphatase may be too high, the pH too acid if the participation of the prostate fluid dominates over the content of fluid from the vesicles. An increase of epithelial cells in the second part of a split ejaculate is an indication that the seminal vesicles are involvd.
3. If an insufficient function of the prostate is the cause of the asthenozoospermia these may be a high viscosity and sometimes a low content of prostatic acid phosphatase. If the cause is a silent prostatitis leukocytes will be found in the first part of split ejaculate.
4. If none of the above named factors can be found, the fault is most probably in the spermatozoa themselves. Sometimes this can be seen in the coloured slides, when a high percentage of abnormal midpieces is encountered

or a great many abnormal tails. This can only be established on faltlessly fixed and stain slides.

5. There still remain a number of cases of asthenozoospermia where no cause can be detected along the lines mentioned above. Doepfmer (1969) found that in some patients with unexplained necrozoospermia the testicular spermatozoa, obtained by taking testicular biopsy, did not show any movement if brought in saline or some other nutritive solution. Testicular spermatozoa will usually show some degree of movement in these circumstances. He used this method to distinguish a certain group of patients with "congenital necrozoospermia".

Relation with fertility

Good forward propulsion of the spermatozoa is not only necessary for reaching the ovum but also to penetrate through the corona and to enter the egg cell. However , low motilitly in the semen is not conclusive of a deficiency in this respect. In all cases of asthenozoospermia the motility should also be studied in the cervical mucus.

Real disturbances in motility are more important in a negative sense for the fertilizing capacity of the semen than low sperm count or poor morphology.

VITALITY

Living cells cannot be stained by supravital stains. These substances are not able to penetrate the cell membranes of living tissue. This is used to find out immobile sperm cells are dead or still alive. In normal semen samples at least 70

percent of the as permatozoa do not take the stain. This is expressed as the semen having a high vitaility.

Pathology :

In normal circumstances there is a relation between the percentage of immobile spermatozoa and the percentage of sperm cells that take the vital stain (Hammen, 1945). found the percentage of stained sperm cells dependant on the pH, increasing in acid enviornment with a rearrence of motility after increasing pH to alkaline. Being motile alive or dead and taking the vital stain are apparently not identical factors.

Relation to fertility

The vitality of spermatozoa in fresh semen may be of importance with the respect to the prognosis of astheno or necrozoostermia. With a high percentage of stained and thus possible dead sperms the chances for a successful treatment are negligible. unforturnately the opposite is not true.

Normal Data

Normal spermatozoa do not clump together. When they meet and touch each other when moving around, there is no sign of adhesion.

Pathology :

In certain semen samples, there appears this peculiar phenomenon that is called agglutination. The spermatozoa stick together either head to head or tail to tail, sometimes in a mixed pattern. This is not yet present in the fresh semen; it develops and increases in the course of time, usually within one hour. In some patients practically all the

spermatozoa in the semen are agglutinated in the end, although the motility is not lost. In the tail to tail type the heads can be seen at the periphery of the clump. swinging frantically from side to side. In the head to head type, the tails can be seen soving outside of the nucleus formed by the agglutinated heads. In the serum of these patients sperm agglutins are present.

Sometimes the spermatozoa are not only adherent to each other, but also to the round cells in the semen, usually the leucocytes. If in the centre of a clump or agglutinated spermatozoa a leucocyte can be seen, this is called pseudo-agglutination. In this, case there are no sperm-agglutinins in the blood.

In samples with a high viscosity the spermatozoa may end up, after losing their motility, lying alongside each other in strings or threads throughout the semen. This string - agglutination is the presence of antibodies in the semonal plasma. The titre of the antibodies is higher when tested in the blood, that in the semen.

As with all immunoglobulin antibodies (Ig), in spermagglutinins several types can be distingushed, of which IgM is involved. In tail to tail agglutination usually mainly IgG is present.

In some patients, there is a high titre of spermagglutinins in the blood and only a small degree of agglutination in the semen. It is probable that in those cases the antibody is IgM (Rumke, 1974). IgA spermagglutinin also occurs, formed locally, but rarely in a high titre.

Relation to fertility

Although the agglutination and other autoimmunological factors are recognized to lower the fertility, a follow up studey by Rumke et al (1974) has shown that a certain pregnancies still do occur. That this happens more in patients with a normal number of sperm cells than in those with oligozoospermia is understandeble. But ther is also a significant reverse relation between the titre of the spermagglutinins in the blood serum and the pregnancy rate.

Formation of antibodies as an autoimmunological phenomenon and especially the presence of spermagglutinins in the blood serum is now generally accepted as a cause for subertility or even fertility. Extensive studies have been made all over the world.

MORPHOLOGY

a. Head forms

In a well stained slide the normal sperm head appears to be oval with smooth contours. In living semen it can be seen that human sperm heads have a large and a small diameter. In coloured slides the heads are lying as a rule with the larger diameter visible and described as so called "front position". A normal head measures 4.5X2.3X1.5 micrometer with a surface size of 8.5 um2. The distal part is more or less clear.This is the cytoplasm, sometimes called acrosome.

The head of the spermatozoa is occupied largely by the nucleus, which is the dense and darkly staining proximal part

of the head. This nucleus is filled with closely packed chromatin, consisting of desoxyribonucleoprotein. the finer structures have been revealed with the electron microscope, but for our purpose it is not necessary to give such an ultrafine description. The proximal part of the head, the borderline with the midpiece, appears as a slightly bent line.

The morphology of the sperm head is determined by four factors.

1. The size (small, normal,big).
2. the relation between length and width (round, oval, elongated.)
3. the shape of the head near the midpiece (normal or pear shaped)
4. the contour (smooth or irregular).

Because these factors may be present independently from each other, the number of possible appearances of a sperm head theoretically is : 3X3X2X2 =36. However, the study from Van Duijn, Jr. et al. (1972) has shown that the percentage of normal sperms is a more sensitive criterion for the evaluation of an ejaculate than the percentage of abnormal sperm heads. This is also the opinion of many other andrologists [Eliasson,1971; Schirren, 1971].

Therefore it is not necessary to note separately all 35 possible sub-groups of abnormal sperm heads.Only a few special kinds of morphology have to be distinguished because of their clinical significance.

1. **Size**. The size of a normal oval sperm head is about 8.5 um2 Less than 7.0 μm2 is too small ; more than 13.0

μm2 is too large. This leaves a fairly large range of normality.

2. **Shape**. In a normal oval sperm head the length this about 4.5 μm and the width 2.6 μm with a ratio of 1:7. If the ratio is 2:0 or more, the sperm head is too elongated ; with a ratio of 1:4 or less, the head is too round. Still, these heads, in common with pear-shaped head forms, are not considered to be pathological, unless the deviation is extreme.

Spermatic stain analysis of human sperm acrosomes

The acrosome reaction of the ejaculated sperm cell occurs either spontaneously of at the surface of the zona pellucida after binding to the ZP3 receptor protein. The sperm head enters the oocyte by endocytosis, decondenses, and forms the pronucleus, which shortly thereafter participates in syngametic approximation with the oocyte pronucleus. Failure to fertilize is thought to occur when the sperm lacks acrosomal enzymes (i.e. the round-headed syndrome or prematurely loses acrosomal enzymes because it undergoes a spontaneous acrosome reaction.[65]

3. Contours. All these heads are only rated as normal if the contours are smooth and regular.

4. Double heads are also observed.

Pathology :

Every semen sample contains a certain percentage of non-oval sperm heads, some abnormal midpieces and abnormal tails. it is impossible to state the exact limit where normality ends and abonormality begins. Not only because there is always a subjective factor involved in the evaluation, but also because it is not known for certain for every type of

non-oval head form whether this is to be considered as abnormal.

ABNORMAL FUNCTION OF THE TESTES

1. The normally sized sperm head may be oval, round elongated or pear shaped; these types are all considered to be normal as long as the contours are smooth.
2. Too small sperm heads, if the size is below 7.0 um2. Extreme examples are the pinheads and the microstrongylostrongylospermatozoa.
3. Too large heads if the size is above 13.0 um^2.
4. large, elongated and tapering head form, the ominous tapering heads described in papers of MacLeod.
5. Amorphous heads are those with irregular contours or with too densely staining nuclei; the last named type is sometimes called abnormal acrosome.
6. Double heads with only one tail.

Even in normal semen a few abnormal head forms are always present. However, sometime a special type of abnormal head form is present in great numbers forming a characteristic seminal pattern with more or less pathological significance.

Teratozoospermia : A high percentage of teratoforms may be the result of fixation or staining (Hellinga et lal. 1973). If technical faults are eliminated, the cause has to be found in the spermatogenetic tissue, because the morphology of the heads does not change during the passage of the sperm cells through the efferent seminal tracts. As soon as the condition of the spermatogenic tissue is damaged by general

factors (fever, malnutrition) or by a local anomaly (varicocele) the number of teratoforms of several kinds and often also the tapering forms increase, together with a decrease in the density and in the motility of the spermatozoa. At the same time, the numer of round cells in the semen, the desquamated cells, tend to increase. This is the "stress pattern of the testes"(MacLeod, 1962).Often this seminal pattern is seen without any known cause. it is considered as a sign of poor condition of the seminal tissue and the increase in output of FSH which is most often found in those cases could be seen as a result of non consumption.

Relation to fertility

In patients with teratozoospermia the fertility is definitely lowered, but it is uncertain whether this caused by the high percentage of abnormal headforms or by the accompanying decrease in density and motility.

Midpiece

The length of a normal midpiece is about the same as the head, with a diameter of about 0-5 micron. The midpiece should be straight with a central implantation in the sperm head. A small droplet of protoplasm on the midpiece near the sperm head is not considered as abnormal.

Pathology

If there is a droplet of protoplasm on the midpiece of considerable size, this is in bulls and other animals a sign of immaturity, possibly caused by a decreased function of the epididymis. It is uncertain whether this also applies to the human.

Relation to fertility

Bent midpieces and more especially the crooked ones near the head tamper with the forward propulsion of the spermatozoa they are therefore to all probability to be considered as interfering with fertility.

A great many bent and crooked midpieces cause a qualitative and quantitative decrease in motility and consequently a decrease in fertilizing capacity.

An abnormality that goes with complete sterility is the elongation and thickening of the midpieces as first decreased by Williams (1950).

Tail

The tail has about ten time the length of a normal sperm head, between 30 and 50 microns. The tail should be straight or only slightly bent. The sheath, a fibrous structure, and other particulars are not visible separate in the normal microscope.

Pathology Tails that are crooked, grossly bent or broken, are considered as being abnormal. Not rare is the occurrence of coiled tails, either surrounding the head or just behind the sperm head. In some patients the percentage of coiled tails increases with the period of abstinence.

Relation to fertility Patients with crooked, bent, broken or coiled tails but still a percentage of normal spermatozoa in their semen have been of proven fertility, although probably subfertile, Patients with a high percentage of short tails are to all probability sterile. This is certain of those with the combination of thickened midpieces and short tufted tails.

Technique : Rated from examination of 200 spermatozoa. A smear was made on a slide, dried in air and fixed with 10% formaline, for aminute, rinsed in distilled water and stained for 2 minutes in hematoxylin.

OTHER CELLS IN THE SEMEN

In human semen a number of round cells are always present. These are of different nature:leucocytes, desquamated cells of the seminal epithelium, also called exfoliated cells, epithelial cells of the walls of the seminal ducts, sometimes cells from the vagina.

LEUCOCYTES

A few leucocytes are normally present. If so, a count should be made with counting chamber. The range lof normality lies below $1X10^9$ /L. For normal semen this means that there are less than 3-5 leucocytes to every 100 spermatozoa.

Pathology

If there are more than $1X10^9$/L leucocytes, a split ejaculate should be examined. In patients with a prostatitis the leucocytes are found in the first part, in vesiculities in the second part and in posterior urethritis in both parts of the split ejaculate.

Relation to fertility

The presence of pus cells is in itself not a hindrance for fertilization. If leucocytes are added to normal semen the motility is not decreased.

However, the presence of leucocytes suggests the existence of a local inflammation and this may well be

accompanied by a decreased function of the infected accessory gland, and thus by a decrease in fertility.

DESQUAMATED CELLS

There is still some controversy about the frequency of finding unripe germinal cells in the semen. It is agreed that spermatids are often present; these cells are easy to recognize. About spermatocytes and spermatogonia there is disagreement.

SPERMATIDS

Spermatids as seen in coloured slides of human semen are about the size of lymphocytes. The nucles is round and dense, the protoplasm stains heavily blue or red. Not more than 3-5 are seen against 100 spermatozoa in normal semen.

Pathology. More than 5 spermatides per 100 spermatozoa are often encountered in semen with a low sperm count. They appear in abundance in relation with pathological conditions, as severe transitory illness, disappearing spontanously within two to three months. This phenomenon has been studied by Barton and Wiesner (1950) and more in detail by Macleod (1950) who found a close relation between the appearance of nucleated round cells, the increase of abnormal headforms and the decrease in motility and density. He called this the "stress pattern" of the testes.

Too many mononucleated spermatids are sometimes encountered without gross abnormalities in the semen, but the presence of multinucleated spermatids is always regarded as a disturbance of spermatogenesis.

Relation to fertility

All in all we may assume that the finding of a great number of spermatids in a semen sample points to the presence of other factors of which it is known that they go with a decrease in fertility.

SPERMATOCYTES AND SPERMATOGONIA

According to Joel (1953),in stained slides spermatocytes have a diameter of 11-19 μ, with a large, single, mostly round nucleus. The protoplasm is clear with small granules and a light perinuclear zone. Spermatogonia are smaller, 5-12μ and and also round. The nucleus is small and darkly staining. The protoplasm is darkly eosinophilic.

Pathology : Cells resembling spermatocytes and spermatogonia are often found as a transitory occurrence in acute distress situations as fever, ischemia, intoxications.

PROSTATIC CELLS

Normally there are no prostatic cells in the semen.

Pathology : There are two possibilitis for finding prostatic cells. The first is after treatment with estrogens. This will not occer often, as the treatment in itself causes ejaculatory impotence. Second : these cells are found once in a while as an artifact, if the patient has produced the semen by massage, after he has been examined by the doctor, this including palpation of the prostate. They appear as clusters of small cells, with inconspicuous nuelei. If the semen investigation is repeated with preceding rectal examination the cell will be absent.

Relation to fertility : None.

ERYTHROCYTES (HEMOSPERMIA)

Normally there are no erythrocytes in the semen.

Pathology : A red or brown colour of the semen is suspicious of the presence of erythrocytes or blood. The diagnosis should be established by microscopic examination but also by the reaction with benzidine.

There may be an external source of origin, a fissure in the frenulum or in the prepuce. If reflux semen is delivered or semen produced by coitus interruptus, it may come from the vagina, for instance during menstruation. If these possibilities have been ruled out, it is likely that there is an internal cause. A fractionated ejaculate should now be examined.

If the erythrocytes are present in the first part of the split ejaculate, the chance is great that the blood comes from the prostate and the patient has to be referred to the urologist. The cause may be a calculus, prostatitis or a malignant tumour.

Most often the erythrocytes will be found in the last portion. This indicates that the seminal vesicles are the site of origin. In 19 out of 20 cases, this a harmless situation and the anomaly will disappear spontaneously within a few weeks or months. Therefore immediate drastic measures as vesiculography and such are not needed. If the hemospermia persists or recurs more than once, the cause must be sought by further, examination by the urologist.

Relation to fertility

In hemospermia the motility of the spermatozoa and thereby the fertilizing capacity is said to be decreased or even absent in most cases.This is especially true for the rare persistent form, in which both the motility and the fertility have been known to improve after surgical or anti-inflammatory treatment. In the far more frequent transitory cases of hemospermia a decrease in fertility has not been proven.

Some definitions in summary

Normality criteria for semen sample :

spermatozoa concentration	>	20 milion/ ml
Motility	>	40% with linear forward progression
Morphology	>	50% Normal (ideal) forms
Viability	>	60% live
agglutination		No
Seminal Fluid	-	Normal apperarence
	-	Normal Viscosity
	-	less then 10^6 WBC/ml.
Aspermia	-	No Fluid
Oligospermia	<	1.5 ml semen
Polyspermia	>	6.0 ml semen
Azoospermia	-	No spermatozoa in fluid
Oligozoospermia	<	20 million sperms /ml.
Polyzoospermia	>	250 million sperms /ml
Normozoospermia	-	20-250 million sperms /ml
Asthenozoospermia	-	Motility < 40% 2 hrs after

ejacula tion or qualitatively insufficient, without good forward propulsion.

Necrozoospermia	-	absence of motility
Teratozoospermia	-	Too high number of abnormal sperm heads.

SPERM FUNCTION TESTS

(a) **Sperm cervical mucus interaction** "The ability of spermatozoa to penetrate the cervical barrier is an important aspect of sperm function that correlates with the fertilizing potential of human spermatozoa" in viva and vitro[67]. In conventional Kremer assay cervical mucus is collected from the female partner during the periovulatory stage of the cycle and carefully loaded into the capillary tube. One end of the tube is then sealed while the open end is inserted into the reservoir of male partners semen. After an incubation period of 30 min. to 1 hr. at 37°C, the capillary tube is removed from the semen and the concentration of spermatozoa counted at intervals along the tube (1,4 and 7 cm). All this information along with measurements of sperm concentration is used to compute the cervical mucus penetration score. Cervical mucus penetration tests provide important information on the first stage of sperm transport to the site of fertilization. What happens to human spermatozoa between the colonization of cervix and initiation of fertilization is largely unknown. In diagnostic terms, the only system available for determining weather human spermatozoa can ascend the

female recovery. Thereafter, the functional assessment of human spermatozoa rests entirely on their competence to participate in the fertilization process itself.

Fertilization is a complete cascade of events and the next step in evaluation of sperm will be to assess interation between sperm and zona pellucida, induction of acrosome reaction and fusion with vitelline membrane of oocyte. These are complicated tests and will not be discussed here as they fall beyond the scope of the present study.

EVALUATION OF OBSTRUCTIVE CAUSES OF MALE INFERTILITY

Patients having normal testicular size and consistency and low ejaculate volumes, sperms density, sperm motility and forward progression should be evaluated to exclude obstructive pathology and varicocele.

RADIOLOGICAL

(A) **Deferentovesticulography** - It is used to study the pathology of seminal duct or prostate by instilling a dye (triiodate hydrosoluble methylglucamin salt at 70% through a puncture at vas. It can identify.

(1) Vas deferens : Enlarged lumen, irregular edges, dilated areas and beaded appearence is usually tubercular obstruction.

(2) Ejaculatory ducts : Unilateral or B/L agenesis can be seen but more commonly provoked by chronic prostatitis that strangles the ejaculatory ducts or provoked by verumontanitis.

(3) Seminal vesicles-Hypoplasia, sclerosis atony (vesiculitis) can be seen.

Findings should be correlated with history, seminogram evidence of urinary tract infection or pus cells in semen, Red cells in semen.

(B) Transrectal ultrasonography

Randal et al. 1993[68] have used transrectal ultrasonography to detect ejaculatoryduct obstruction and have advocated this as a good procedure and concluded that

ejaculatory duct dysfunction has been under diagnosed in the past. This procedure can also be used for the examination of prostate, seminal vesicle.

(C) Scrotal ultrasonography

This is a reliable method for identification of varicocele although venography is the most specific but invasive.

Hydrocele- is sharply depicted as an anechoic fluid collection around the testis between the two layers of the tunica vaginalis. *Hematocele* - in comparison presents a complex fluid collection of variegated echogenicity.

Infection - results in an altered echo pattern of the epididymis, which is thickened, and also of the testis if involved. These areas are usually hypoechoic with ill defined margins. A secondary hydrocele or an abcess, usually extra-testicular, may be in association. The latter appears as a localised cysitc area of complex echogenicity[69].

Testicular tumors - appear as intra-testicular hypoechoic areas, occasionally with mixed echo patterns, having irregular but well defined margins, surrounded by normal testicular parenchyma. Cystic areas and calcification may also be

encountered[69].

Immunological Tests :

Immunological factor was studied by observing the agglutionation of sperms in seminal fluid by testing for antisperm antibodies in the serum of patient.

Antisperm antibodies :

Serum dilution (0.5ml in each test tube) is done with normal saline 1:10 dilutions upto dilutions are done. One tube is set with only normal saline to serve as control. After liquifaction of semen,an equal amount (.5ml) is mixed in each tube. All test tubes are incubated for 1 hour at 37° C The semen is examined on a slide in microscpe and it is examined for loss of motility and agglutination of sperms to form clumps.This is taken as positive for presence of antisperm antibodies.

ENDOCRINE EVALUATION

Following 3 hormones were assessed by ELISA.

Hormones assessed and their normal values

HORMONE	Normal values
FSH	1.0-14.0miu/ ml
LH	7-7.4 miu/ ml

Testosterone, T_3 ,T_4,TSH & Prolactin

To compensate for the pulsatite releasé of the gonadotropins, following method for collection of sample has been recommended and used.

Blood samples were taken at 20 minute intervals from the same or different vein and mixed together and analysed. A normal FSH in presence of semen abnormalities is a good

guide to the presence of obstructive pathology.

If serum LH and FSH concentrations are increased the diagnosis is primary testicular failure. These men generally have low testosterone levels, small testis and azoospermia, but may have oligospermia and normal testosterone levels[72].

In some infertile men LH and testosterone levels are normal but FSH levels are elevated this is because FSH signifies the state of seminiferous tubules[73] which are more sensitive to damage than leydig cells. A selective increase in FSH can also signify a FSH secreting pituitary tumor. Consequently Patients with azoospermia having elevated levels of FSH fall into 3 categories on testicular biopsy,seminiferous tubule hyalinization, sertoli cell only syndrome and germinal cell arrest, all untreatble conditions. Thus finding of elevated FSH with azoospermia usually is an indication to proceed no futher in investigation or therapy.

The estimation of LH and testosterone level in plasma provides evidence that intertubular areas are also involved in the pathological process. As damage to seminiferous tubules becomes severe, an increasing proportion of patients show an elevated FSH and a low testosterone.

Finding of a low testosterone should also prompt search for hypogonadism in which case Gonadotropin level would also be low. It has already been mentioned that normal FSH with oligo-azoospermia can be because of testicular cause but should also prompt investigation to rule out obstruction in the male genital tract.

Serum prolactin levels are measured in men with sexual dysfunction, decreased libido or delayed adolescence because these symptoms are common with prolactin producing pituitary tumors.

Elevated testosterone levels are usually a consequence of increased serum hormone binding globulin level, as in hyperthyroidism[74]. Testosterone production may be increased however in patients with LH producing pituitary tumors or HCG Producing neoplasms or with mutation in androgen receptor that disrupts testosterone negative feedback.

MALE ACCESSORY GLAND INFECTION :

Foollowing criteria recommded by WHO was used

History and Physical signs :

* a history of urinary symptomps (dysuria, urethral discharge, hematuina, increased frequency or difficulty in voiding)
* a history of epididymoorchitis
* a history of painful ejaculation.
* a history of sexually transmitted disease, thickened,tender or cystic epididymes on clinical examination
* thickened vasa deferentia on clinical examination
* Postinfectius /trauma scars on initial examination
* lymphadenopathy
* abnormal prostate on rectal examination
* Palpable seminal vesicle on rectal examination

Urinary or Prostatic signs

* increased leukocytes on urine analysis
* significant bacteriuria ($>10^5$ /ml) on urine analysis

Ejaculate signs

* abnormal apperence of semen
* abnormal viscosity of ejaculate
* Elevated WBC ($>10^6$/ml) in semen sample

Any combination of 2 or more signs, symptoms from the 2 categories were sufficient for diagnosis. In the absence of any signs or symptoms there had to be at least 2 ejaculate signs for diagnosis.

TESTICULAR BIOPSY

Evaluation of testicular biopsy is a direct method of evaluating the state of seminiferous tubules in male with infertility. Studies by Zuckermann et al. showed a direct relationship between seminiferous tubules and sperm counts. Rodriguez et al. showed direct relationship between sperm counts and elongated spermatids on biopsy[70].

Testicular biopsy is also useful to demonstrate partial or complete obstruction. In the former, the late spermatid count per tubule cross section is inappropriately high. Analysis of testicular biopsy specimen show the direct relationship between germ cell counts and serum FSH and testosterone production and are thus useful for diagnosing Sertoli cell only syndrome, Klinefelter's syndrome[71]. Testicular biopsy can identify the type of testicular damage. Evaluation of seminiferous epithelium is performed at low magnifiction to assess the overall state of spermatogenic process. Each individual cell type is sought and some assessment of this number is made in comparison

to normal values. Abnormal cell types are sought.The degree of testicular development is compared to the chronological age of the patient and his pubertal status.

Categorization

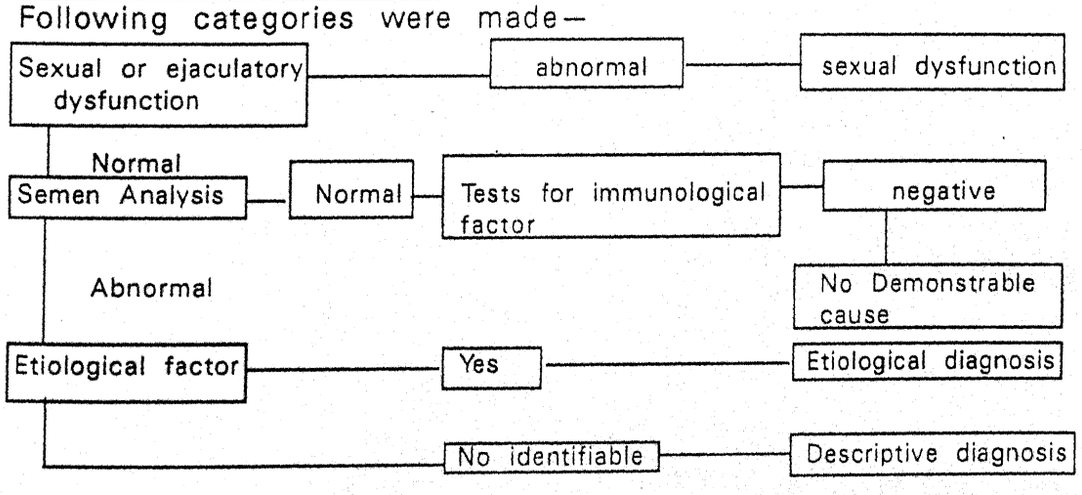
Their is no universally accepted categorization but the following is recommended -

CATEGORIZATION OF TESTICULAR BIOPSY FINDINGS

	Category	Appearence	Semen FSH levels
1.	Obstructive azoospermia	Near normal histology	Normal
2.	Hypospermatogenesis	All stages of spermatogenesis Present. Number of germ cell depleted. Peritubular fibrosis in severe depletion	Normal or elevated
3.	Sertoli cell only syndrome	No germ cell. Sertoli cells only	Elevated
4.	Germinal cell arrest	Cessation of spermatogenesis at Primary spermatocyte or spermatogonia stage	Normal or Elevated
5.	Seminiferous tubule hyalinization	Fibrotic or hyaline outline of tubules	Elevated
6.	Immature test	Testicular development retarded in relation to chronological age	Low

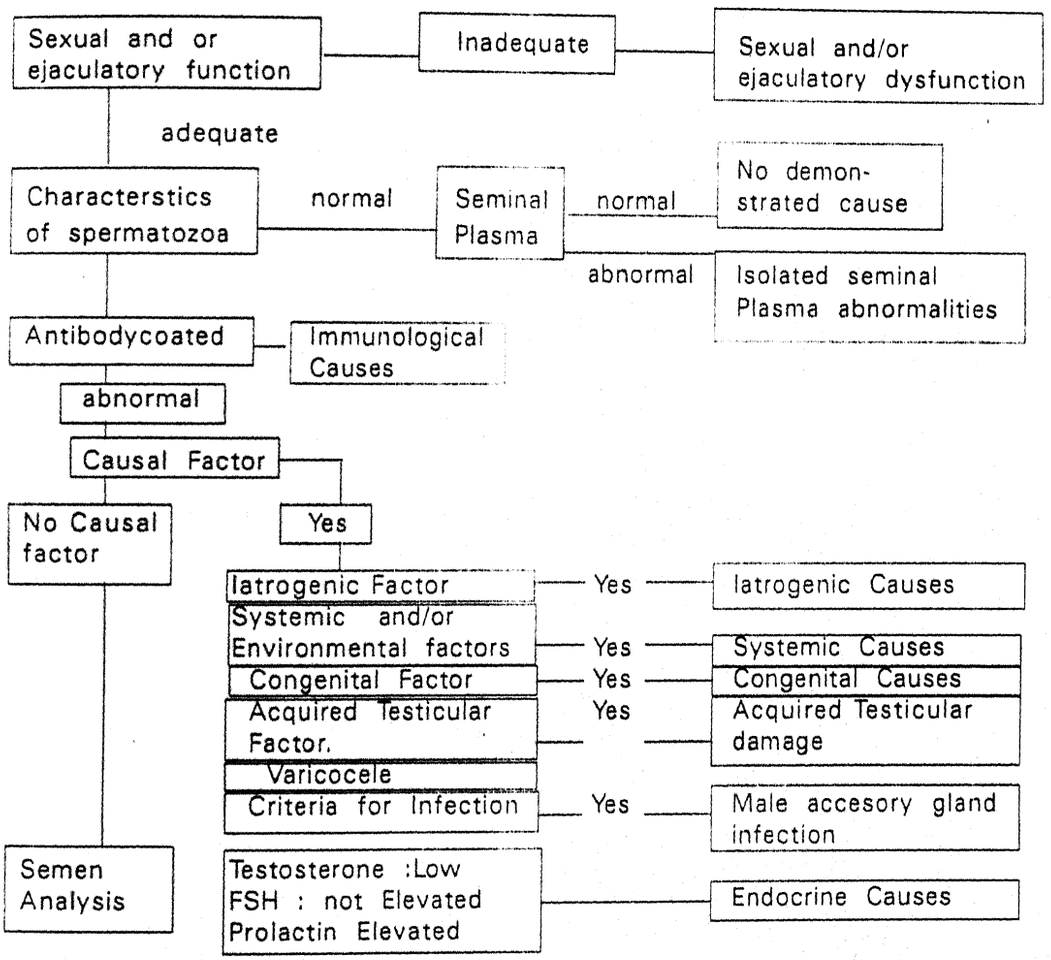
DIAGNOSTIC CATEGORIES :

Following categories were made—

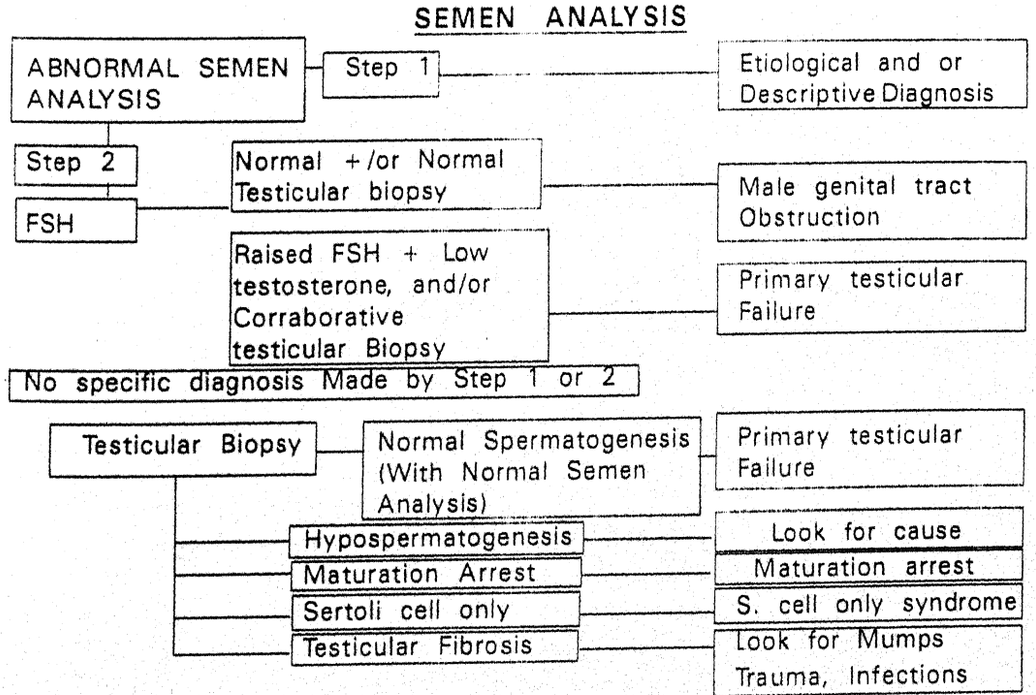


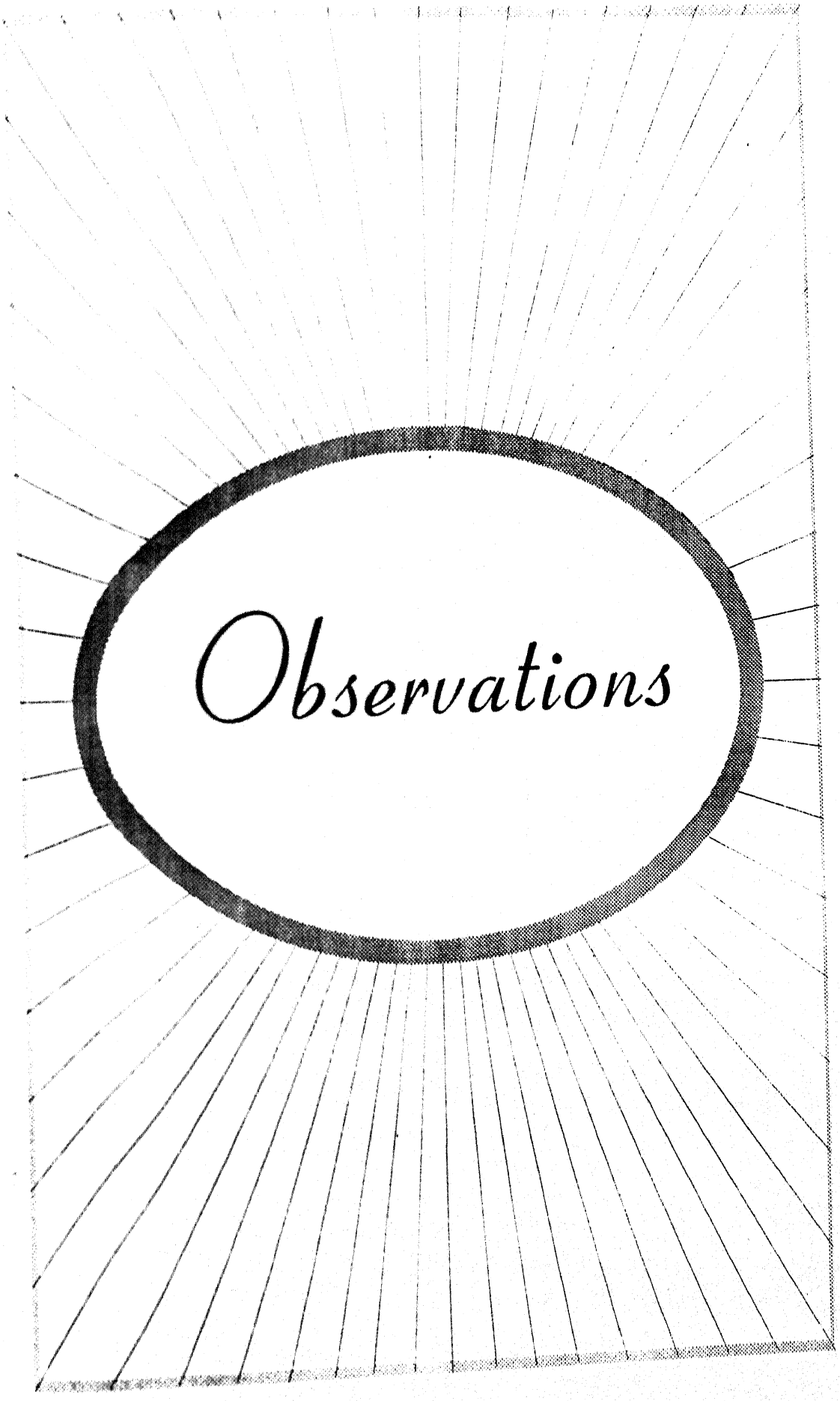
PATIENT WORK UP PLAN

Each Patient was worked up according to the following



SEMEN ANALYSIS




Observations

OBSERVATIONS

The initial part observations will be grouped according to the following headings —

HISTORY :

1. Age
2. Duration of marriage
3. Primary or secondary infertility
4. History of any relevant systemic illnesses
5. History suggestive of male genital tract infection according to criteria mentioned previously.

PHYSICAL EXAMINATION:

General

Systemic

Local

Semen analysis

Antisperm antibodies

Testicular biopsy

Endocrinal investigations

A total of 78 couples attended the Infertility clinic, of which 34 (43.59%) showed major abnormality in their clinico-laboratory profile.

AGE :

The table given below shows the age parameters of subject.The table shows the number of subjects in each age group-

Table:1 AGE DISTRIBUTION OF STUDY SUBJECTS :

AGE (in years)	No. of patients n = 78	% of total
< 25	17	21.79 %
25- 30	49	62.82 %
> 30	12	15.38 %

This it is evident that the majority of subjects belonged to the age group of 25-30 years. The mean age of patients was 27.42 $\pm$ 3.04 with youngest age being 20yrs old & oldest being 42years old.

Duration of marriage :

The following table shows the duration of infertility in the subjects studied.

Table: 2 DURATION OF INFERTILITY IN STUDY SUBJECTS

Duration of infertility	No of patients	% of total
< 2yrs	4	5.128 %
2- 4yrs	36	46.15 %
5- 7yrs	21	26.92 %
8- 10yrs	11	14.10 %
> 10yrs	7	8. 97 %

This is evident that very few 4 (5.128%) of the patient reported for infertility within 2yrs of their marriage. 2-4 years after marriage maximum number of patients 36 (46.15 %) reported into our study. Similarly number of patients reporting after 10 years of marriage was 7 (8.97%).

Rural /Urban :

36 (46.15 %) Patients were from urban areas & remainder 42 (53.84 %) from rural areas.

Primary / Secondary infertility :-

In our study the percentage of males complaining of secondary infertility was very small. Only 12 cases (15.38%) cases out of total 78 cases included in the study had secondary infertility.

Symptomatology: The following table shows that the number of patients giving history suggestive of various diseases in the past or at present.

Table - 3

Disease	No. of Patients
Urinary tract infection	14
Hypertension	2
Diabetes	1
Tuberculosis	5
Mumps	3

Physical Examination & Routine investigations :

Physical examination showed 5 patients showing evidence of pulmonary tuberculosis on chest xray i.e. evidence of fibrosis, cavitation or both. These patients also gave history of prolonged low grade fever & cough. 2 patient has history of hemoptysis further investigations of these patients confirmed the presence of pulmonary tuberculosis in these 5 patients. Out of these 5 patients, 3 patients also gave history of urinary symptoms.

URINE Analysis : A total of 14 (17.98 %) of patients showed evidence of urinary tract infection. Out of these 10 had given a history of urinary symptoms while a history was not obtainable in remaining 4 patients but their urine examination showed pus cells.

Genital examination: The size of the testes was especially noted. The criteria used was for diameter of the testes - the normal value for such a measurement is 40 - 50mm. 5 patients showed the diameter below 40mm. & were considered abnormal. Out of these 5 patients with subnormal testicular size, 1 patient showed normal semen analysis, 2 showed oligospermia & remaining 2 showed azoospermia.

One patient showed illdefined secondary sexual characterstics & none of our patient showed any significant deformity of penis but one patient showed poorly developed scrotum.

Sexually transmitted diseases : 3 patient had confirmed sexually transmitted diseases; all of them suffered from syphilis (VDRL positive).

But if symptoms suggestive of sexually transmitted diseases like urethritis, urinary tract infection, history of discharge from penis, pain in genital organs, ulcers was taken into account, the number grew much higher.

TABLE - 4 DISTRIBUTION OF STD RELATED SYMPTOMS:

Particulars	Total No. of Cases	Cases with normal semen	Abnormal semen
Confirmed STD	3	1	2
STD (not confirmed)	18	9	9

Semen analysis :

34 Cases (43.59%) of the total number of 78 patients who entered the study show abnormal semen analysis.

The table given below shows the different categeries of abnormal semen & the distrubution of cases between them.

Table-5 Distribution of cases according to semen abnormalities

Semen abnormalities	No. of cases	%of total cases	as%of male showing abnormal semen
Abnormal semen	34	43.59 %	
Azoospermia	9	11.54 %	26.8 %
Oligozoospermia	20	25.64 %	58.8 %
Asthenozoospermia	20	25.64 %	58.8 %
Pus cells	21	26.9 %	61.76 %
Borderline	2	2.56 %	5.88 %

Thus abnormal semen was found in 43.59 % of total study subjects.Out of these 9 (11.54%) cases showed azoospermia, oligozoospermia was seen in 20 (25.64%) cases. Asthenozoospermia was seen in 20 (25.64 %)cases Out of these 15 Cases also had oligozoospermia.Borderline Semen abnormality was noted in 2 (2.56 %) Cases. It is also noted that all cases showing oligozoospermia were not having poor motility.Out of 20 asthenozoospermia patient, 5 cases had normal semen count while 15 had oligozoospermia.

Pus cells in semen were seen in 21 (26.9 %) cases, of these 10 cases showed some other abnormality of the semen.

2 of the cases showed borderline initial semen analysis i.e. it was difficult to determine whether the subject semen was abnormal or normal because of varying semen characterstics on the either side of normality on various semen analysis. Details of the data will be given futher on.

TESTICULAR BIOPSY :

Testicular biopsy was done in 3 cases out of total 78 cases. The results of the biopsy showed maturation arrest of both testes in 2 cases & testicular atrophy in one case. Semen analysis of these subject had shown azoospermia with asthenozoospermia.

Endocrinal evaluation: Endocrinal evaluation was done in 8 cases. All these cases had abnormal semen examination as endocrinal evaluation was done only in those patients showing abnormal semen examination.

Table - 6 Endocrinal evaluation of 8 study subjects.

Hormone	No.of Patients	Elevated or borderline elevated	Normal	Depressed
S. FSH	8	3	5	0
S teststerone	5	-	2	3
Serum LH	5	3	2	0

For getting an idea about the probable etiological factors responsible & various contributing factors. It is essential to correlate the findings of all aspect of the investigation.

The table given below shows the details of findings of all relevant investigations in 15 cases in which it was possible to conjure or confirm the etiological factors responsible or contributing significantly.

Table : 7 Finding in investigation of 15 males of infertility with abnormal semen analysis

S.No.	Semen analysis	Serum Testosterone	Serum FSH	Serum LH	Testicular Biopsy	History Suggestive of STD	Confirmed STD	Pus Cells in Semen	Other findings
1.	Azoospermia	-	-	-	-	Present	Absent	Present	H/O Mumps
2.	Azoospermia	depressed	-	elevated	B/L maturation arrest	-	Absent	Present	Varicocele present
3.	Azoospermia	-	-	-	-	-	Absent	Absent	-
4.	Azoospermia	-	-	-	-	Present	Absent	Absent	-
5.	Azoospermia	depressed	elevated	elevated	B/L testicular atrophy	-	Absent	-	Sec. sexual characterstics absent & H/o mumps
6.	Azoospermia	Normal	-	-	-	-	Absent	-	Pulm. TB
7.	Azoospermia	Normal	-	-	-	Present	Confirmed	-	-
8.	Azoospermia	-	elevated	-	-	-	Absent	Present	-
9.	Azoospermia	depressed	elevated	elevated	B/L maturation arrest	-	Absent	-	Systemic hypertension
10.	Oligozoospermia	-	Normal	-	-	Present	Absent	Present	Pus cells in urine
11.	Oligozoospermia	-	Normal	Normal	-	Absent	Absent	Absent	H/O mumps
12.	Oligozoospermia	-	Normal	Normal	-	Present	Absent	Present	Pus cells in urine
13.	Oligozoospermia	-	Normal	-	-	Present	Confirmed	Present	-
14.	Oligozoospermia	-	-	-	-	-	Absent	Present	-
15.	Oligozoospermia	-	Normal	-	-	Present	Absent	Present	Pus cells in urine

The table revealed that out of 15 cases, 7 cases revealed some obstructive pathology as a cause. Out of these 7 cases, 3 cases showed male accessory gland infection & two cases showed confirmed STD & one revealed evidence of pulmonary tuberculosis & one also revealed pus cells in semen & one case also gave history suggestive of mumps.

Out of 18 cases giving history suggestive of STD, only 3cases had confirmed STD, of which 2 cases revealed major semen abnormality & one revealed pus cells in semen

9 cases revealed azoospermia, of which 3 cases also showed disturbed hormone levels suggestive of primary testicular failure. Of these 3 cases with primary testicular failure, one was hypertensive & one gave history of mumps with failure to develop secondary sexual characterstics. History of mumps was present in three cases in past, of which too were azoospermic & one was oligozoospermic. One case with azoospermia with primary testicular failure also revealed varicocele on examination.

STATIC AND DYNAMIC TRENDS IN SEMEN ANALYSIS (INTER AND INTRA PATIENT VARIABILITY)

34(43.59%) of patients showed abnormal semen examination. The mean sperm count in this group was 5.5 millions/ml while in the patients showing oligozoospemia, the mean sperm count was 10.2millions/ml. In the patients, showing poor motility asthenozoospermia, the mean motility with linear forward progression was 21.2%. 44(56.41%) of patients did not show any abnormality in the semen examination. The mean sperm count in this group was 70.2± 8.2 millions/ml.

Since a minimum of 2 semen samples were taken each subject .The variability between the 2 or more semen samples of subjects showing normal or abnormal examination was studied.

INTRAPATIENT VARIABILITIES IN SEMEN ANALYSIS

No two or more semen analysis of the same patient or for that matter any semen analysis of any of the patients was exactly similar.

But considering only intrapatient, it should first be stated that we took at least 2 semen samples of all patients irrespective of the fact that wether the first sample showed normal or abnormal examination.

In terms of sperm count, it was seen that the second semen sample was better than the first sample in 27.6%(22) cases, with upgrading from azoo-oligo-normospermia or anywhere in between for persons showing abnormal semen examination and an increase in sperm concentration for patients showing normal initial sperm examination. Similarly a degradation was observed in5.6% cases only.

Also None of patients showing azoospermia had spermatozoa even in the second sample.

In terms of motility also the second sample was better than the first in 19 cases and worst in 11 cases.

If we consider only semen samples of all subjects we analyzed .the number of patients whose intersample variability was much that the 6 samples were categorised into different categories(oligo,azoo, normospermia)12(65%) patients out of our total of 78 patients showed such variation in any one collection of semen compared to any other.

Thus it is seen that in 6 patients the diagnosis changed from azoospermia in 2nd in 10 others from oligospermia to Normospermia or vice versa.

2 patiants showed extreme variabillty in quality of sample in the first two and in subsequent also.

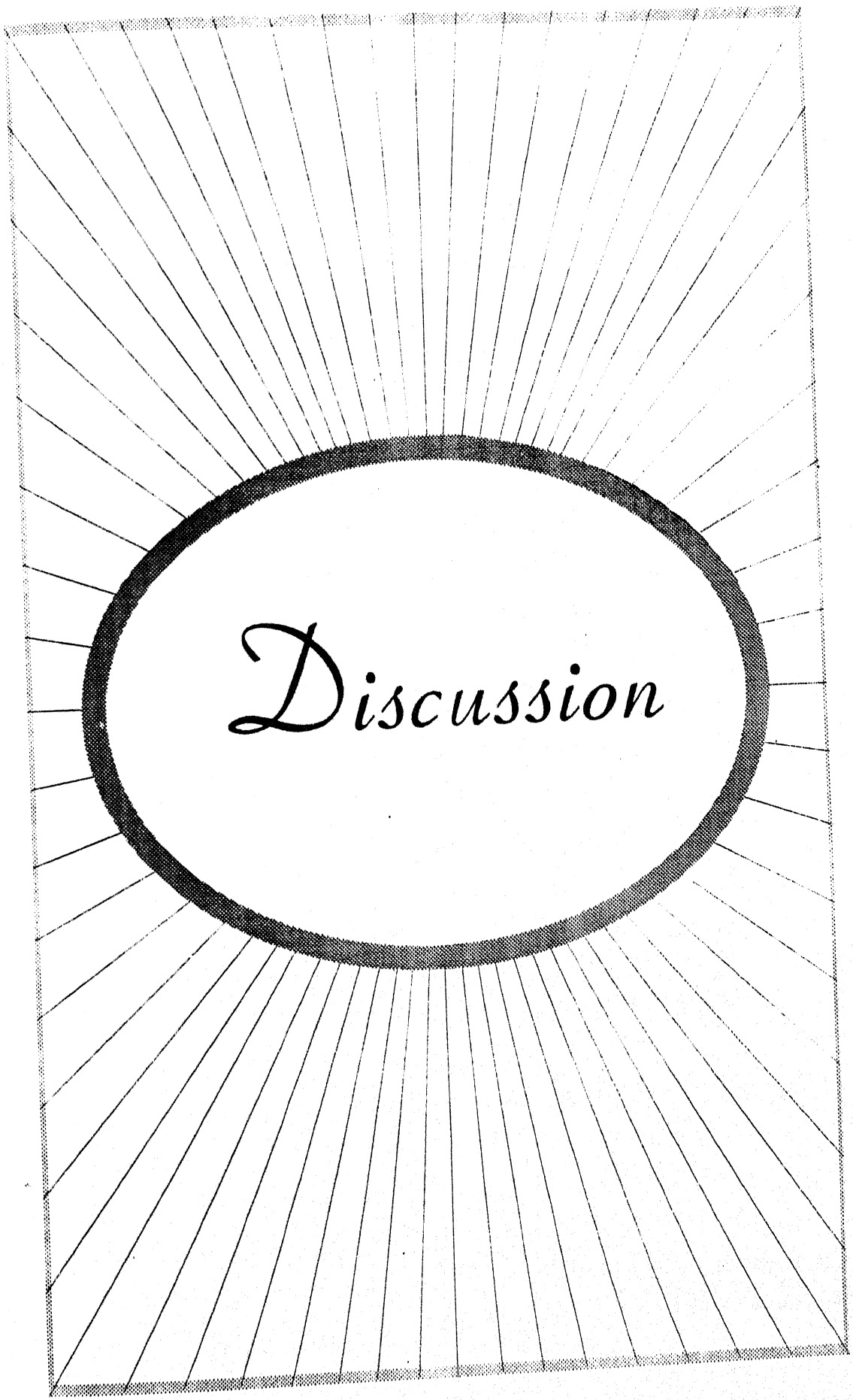
Thus this patient showed extreme variability in sperm concentration and a lesser variation in sperm motility in multiple semen analysis.

INTERPATIENT VARIABILITY

This has already been mentioned before. Semen characterstics were highly variable in all patients with infertility.The range of sperm count was 0-126 millions per ml.

ANTISPERM ANTIBODIES

In none of our subjects,were antibodies detected in semen or serum of male.


Discussion

DISCUSSION

The first point to be noted in our study is that out of 78 males of an infertile couple investigated 34 males, (43.59%) showed abnormal sperm counts.Thus 43.59% males of infertile union in our study showed abnormal semen as a cause of infertility.Data from investigation of the female by coinvestigator shows that in out of 34 couples in 24 couples some defect was also found in the female partner. so a male cause only was found in 10(13.54%) of all the cases.

These data can be compared with data available from other places. The W.H.O study of the standardised investigation of the infertile couple[76] has done comprehensive study and collected data from all over the world about the factors involved in infertility.

In developed countries, the male factor is found in 43% cases, a male factor only was found in 22% of couples. Data from Chandigarh states the male factor in 24% cases, male factor only in 13% cases and both the partners involved in 25%.

Thus in our study abnormality was found in male in a comparitively very high percentage of cases.43.59% as compared to 22% in Chandigarh respectively, M.C Bansal et al has given a higher percentage for male involvement as 57.4% from Meerut, India. Also in our study both partners were involved in 30% cases which is considerably more then 21% and 25% of values given for developed countries and Chandigarh respectively. Our figure has in between data from Africa which found both partners involved in 35% of cases & in developed voluntreer.

The reasons for this differance can lie basically in the quality of population studied. Bundelkhand region is specially recognised as a very backward region both economically and socially and this

is projected in high prevalance of infectious diseases specially tuberculosis here. The nutrition in this malnutrition and anaemia. Thus infections and malnutrition mean that lager proportion of population is diseased which is reflected in our data by large proportion of persons of both sexes affected by disease.

Our data resemble more closely to Africa with respect to couples in which both partners are affected,35% in Africa and 30.16% in our study. this is expected as most of Africa is an underdeveloped region like this region.

When age parameters are analyzed it is seen that the maximum number of patients seeked medical attention in the age group of 25-30 years 49% and between 2-7 years of marrige 73%.The age group of 25-30 years is also the age of maximum reproductive caoacity . It is also seen that the mean age of persons entering our study was 27.42±3.04 years .Which seems rather high when it is taken into account the trend of marriage at early age in India specially among the poor classes (most of our cases were from low socio economic strata) This may reflect the tendency of couples specially males to present themselves for investigation quite late because of hesitation, taboos and a misconception that female is mostly responsible for fertility .

The majority of patients were of primary infertility (88%) W.H.O has given this figure as 84%for Asia and 84.62% for developed countries.These data differ a lot from Africa where primary infertility forms only 40.8% of the total infertile couples.

The mean age of primary infertility patients was much earlier i.e. at 27.7± .84 years in comparison to couple with secondary infertility who tries to have a baby for longer time before presenting themselves for investigation.

The low incidence of secondary infertility in our study may not

reflect its actual incidence. Couples with one child who subsequently became secondarily infertile are more to adapt to this situation and not seek medical attention then a couple who never has conceived.

A survey report by W.H.O showed that 11% of secondarily infertile women were interested in therapy compared to 40% the primary group. For further discussion we will put all the investigations in a proper perspective.

In our semen analysis the major abnormality was oligoasthenozoospermia in 36.7% of our cases forming 58.8% of all semen abnormalities .Next in line was azoospermia in 11.54% of our cases and responsible for 26.5% of all semen abnormalities.

In our study we have found that low count and poor motility travel almost in parallel in all 15 cases but in 5 case showing oligozoospermia the motility was normal & 5 cases with asthenozoospermia was with normal sperm count. Thus count and motility may be reflector of estimates of the same thing. Divergence of these 2 parameters may indicate a specific defect.In our case, it was maturation arrest of spermatids in the testes in 2 cases showing divergent trends & one case showed testicular atrophy. Similar view has been expressed by Freund 1962 and Elliason 1975.

In our study we did not find any patient with significantly abnormal sperm morphology.W.H.O in its worldwide study gave this figure to about 0.6%.We could not find any case of abnormal sperm morphology probably because of our small study size. This incidence of abnormal sperm morphology is much less frequently diagnosed than in the past this is because of much better understanding of normal variations in sperm morphology.Gordon et al.1965 have shown that normal spermatozoa vary typical for most biological data.

A very high incidence of pus cells were found in semen in our study (26.9%) which may reflect the increased prevalance of genital infection in this region,pus cells is a good indicator of genital infection as a cause of infertility is expressed by the fact that patients with significant pus cells in semen had abnormal semen quality.

A very important aspect of male investigation is finding out the etiology of infertility which is different from just finding out that a particular infertile male has abnormal semen quality. As mentioned by us in material and methods the W.H.O [76] has laid standard guidelines for the standardized evaluation of the infertile couple . and recommended that an infertile male be provided with 2 diagnosis (1) the descriptive diagnosis which has been dealt with earlier and (2) etiological diagnosis.

We have found (Idiopathic)primary testicular failure (Maturation arrest) in 3 out of 78 cases (3.84%) This is low in comparison to the worldwide data provided by W.H.O which published a figure of 10% for developed countries 7% for Africa ad 8% for Chandigarh city in India [76].

Our study also provided for evaluation of obstructive azoospermis.We have estimated that at least 7 out of our 78 cases were having obstructive causes which came to about 9%.The W.H.O has given a 1% for the world average and 4.2% for Africa[76].

However our results in this aspect have to be interpreted with caution. The diagnosis of obstructive azoospermia is not easy.Comhaire et al. state that if testicular volume and FSH is normal, testicular biopsy is needed to reach a confirmed diagnosis[77]. Testicular biopsy was not done in all our cases to confirm the diagnosis and only an estimate was made.But Joel.C.A has also

stated that in patients with complete azoospermia the cause is an obstruction somewhere in deferent ductules,It is rarely that one finds completely normal spermatogenesis in the biopsy.The much more specific methods for detecting obstruction in the male genital tract like deferento vesiculography, transrectal ultrasonography were not performed in our study.

However all our cases suspected of having obstruction had low to azoospermia on semen analysis.

3(3.84%) of our cases satisfied the WHO criteria for diagnosis of male accessory gland infection & all 3 cases was suspected to be having obstructive pathology also, so this group was not exclusive.Confirmed STD was found in 3(3.84%) cases. .

Since these groups are overlapping it would be important to consider total STD related causes which would include confirmed STD.Two cases of azoospermia was caused by STD.Thus 9 cases (11.54%) of cases in our study had STD related causes for their infertility.

Three case had history of mumps in childhood and of which two were azoospermic & one had oligozoospermia. If we include this the total load of infection related causes would be in 24(30.77%) of cases. This figure is higher than WHO published reports of 8.9% for total average of various regions of the world .It also gave this percentage as 14.3% for Tunisia in Africa.

Our results thus reflect a heavy load of infection as a factor for male infertility. This is not surprising as the incidence of tuberculosis and other infections is much higher in this region.

We found 1(1.2%) case of varicocele related infertility and none due to congenital causes.Varicocele has been considered a very important cause of male factor infertility in studies worldwide ranging from 2.2% to 11% in Chandigarh and developed countries

respectively.M.C Bansal et al. from their study in Meerut gave this percentage to be 2.8% only the reason for these differences could be either a true lower incidence of variocele in our country or lower index of suspicion for this easily treatable cause of infertility or small size of our study .

Congenital abnormalities have also been given in literature to be present in 6-15% of infertile males .

Lastly the most perplexing part of etiology of male inferlitily were cases in which no etiological factors whatsoever could be identified in the presence of definite semen abnormalities, 64.7% (22 out of 34) of cases no etiological factors were identified and were put into the category of "no demonstratable cause" of WHO. this figure in WHO. study was 49% from the developed world, 46% from Africa and 73% from Chandigarh. A higher percentage than developed world or Africa in our study would not neccessarily mean that we were able to identity etiological factors in a compartively higher percentage of patients. The difference was more likely, possible because our criteria were much less strict and a final diagnosis was made in lesser number of cases, i.e. in many of our cases the world over male infertillity is a much less understood problem and in majority of cases the exact etiology remains unclear even after comprehensive testing.

We did not find Antisperm antibodies in serum or semen of any of our subjects. This was because we have used a much less sensitive biological method. Newer methods like the immunobead test, act to detect secretory antispermatozoal IgA, SPM test, SCMC test. Various investigators have found antisperm antibodies in fair number of people. J. Kumar and S. jager in a study of 5000 infertile couples found immunologically disturbed interaction in 5% of couples.

Coming to infertile couples in which males showed a normal semen examination, many aspects emerged. In general the second semen sample was better than the 1st. FH comhaire et al working on the behalf of the WHO task force has found similar results [75].

We think that one reason for this was probably patient education. It has already been mentioned that abstinence increases sperm counts, also we observed that patients were much confident in giving samples for the second time than first[78]. Thus the second sample probably was collected in more efficient way, without loss much semen and with good volume. All these factors could have caused this difference in semen quality.

Another very important aspect we want to highlight is the importance of evaluating atleast 2 and if need arises more than 2 semen samples. This is stressed by the fact that 20.5% of our patients at some time gave a sample which was categorized into different category when compared to any other sample given by him. WHO. also recommends at least 2 semen samples to be examined.

In our study the average sperm count of persons whose semen was categorized as normal was 70.2 $\pm$ 8.2 millions/ml. . This low sperm counts could be because of many factors like the heat, wearing of tight langots and dhotis, high incidence of smoking and tobacco chewing habits, general malnutrition and high incidence of exposoure to chemical pesticides in this predominantly rural farming society.

However it was not possible to compare the incidence of these habits' and exposoure among persons showing semen abnormality and those not having normal sperm count because of small size of our study.

Next in line would be social factors. Although because being abstract we could not measure these factors but it was clear that

male, specially the uneducated ones are very hesitant to present themselves for investigation before their wives. Only when no abnormality was found in wives would some of them volunteer for investigations. But it was also observed that education was important. After taking these males into confidence, follow up was not difficult. We even had few instances when male of an infertile union married again and only when this union was barren, he consented to be investigated, but even these case are amenable to patient education and social support.

And finally the most important aspect of a diagnostic work up of a infertile couple would be to offer them fruitful treatment. Idiopathic testicular failure 3.9% is untreatable. These would have to be offered donor insemination or adoption. Donor insemination is now popular and has reasonable pregancy rates of 10-15% per month over the first 6 months[79]. Approximately. 50% of women are pregnant by six months.

However, latest advanced techniques may be offered to these patients also. Silber S.J. has reported 4 successful pregnancies out of 38 in such patients by testicular sperm extraction and ICSI. [80,81] This technique can also be offered to other types of azoospermia [82] Testicular atrophy by mumps also falls in the category (3.84%).

Male genital tract obstructions and male accessory gland infections are potentially treatable by vasoepididomostomy, implantation of spermatoceles, microepididymal sperm aspirations, ICSI, MESA[83] , TESE[84] and IVF can be offered to these patients. The last 2 procedures have better success rate than the initial 2. . Thus 30.7% of our infertile males are potentially treatable. Many of our cases in which no demonstratable cause was found could ultimately land in this category. However with new surgical advances repair procedures are also giving good results.

In our study impotence, failure of ejaculation and retrograde ejaculation were uncommon causes of male infertility.

Thus in our study all of the men seen for infertility had abnormal semen quality with eitiology identifiable in a few cases


Summary
&
Conclusion

only.

SUMMARY AND CONCLUSIONS

The present study was conducted in the Department of Medicine, M.L.B. Medical College, Jhansi to study the 'THE EXTENDED CLINICO LABORATORY PROFILE OF MALE PARTNER OF INFERTILITY IN BUNDELKHAND REGION'.

78 patients were included in the study.

34 (43.59%) of patients showed abnormal semen analysis.

Most of the patients belonged to the 25 to 30 year age group with the mean age of 27.42 $\pm$ 3.04 years.

Only 12(15.38%) of patients belonged to the secondary infertility group.

18 (23.07%) of patients had history suggestive of STD and 3 (3.84%) had confirmed STD.

11.54 of all patients showed azoospermia on semen analysis and 25.64% showed oligozoospermia.

A comparitively high percentage of cases 24(30.76%) showed a defect in both partners of a couple.

26.9% of patients also showed pus cells in semen examination.

3.84% of patients showed idiopathic testicular failure and 9% of cases satisfied criteria for male accessory gland infection.

Thus a high percentage of patients showed abnormality reflecting the high incidence of infectious diseases in our region.

Average sperm count among persons with normal semen analysis was also comparitively low at 70.2$\pm$8.2 millions/ml. (Compared to other regions in the world on the basis of available data).

This probably was due to hot climate, high prevalence of smoking and tobacco chewing and wearing of tight dhotis and langots in this predominantly rural region.

When our results in this region, which is a socio-economically backward region as comapared with other regions from the world on the basis of available literature, our findings match most closely with those of underdeveloped regions of the world. This was reflected in our findings.

Many patients (20.5%) showed a wide variation in semen quality. Thus at least 2 semen analysis or sometimes more should be done for every infertile males before being declared infertile or subfertile.

Social factors like illiteracy, hesitation and ignorance are sometimes major hurdles in getting the male's consent for his full evaluation simultaneously with the female, and proper patient education and councelling should be a vital part of any infertility programme.

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MASTER CHART

S. No.	Patient's Name	Age (Yrs)	Rural/ Urban	Primary /Secondary	Duration of Infertility (Yrs)	Major Abnormality
1	Pramod Kumar	35	Rural	Secondary	8	Pus cells in semen
2.	Jeetendra	27	Urban	Primary	2	Pus cells in semen Oligo asthenozoospermia
3.	Ram Swaroop Kushwaha	42	Urban	Secondary	10	Pus cells in semen VDRL + Ve
4.	Gulab	23	Rural	Primary	8	Normal
5.	Parshuram	25	Rural	Primary	7	Oligo asthenozoospermia
6.	Rajkumar	24	Urban	Primary	5	Pus cells in semen Oligoasthenozoospermia
7.	Sandeep	34	Uraban	Secondary	2	.
8.	Pappan	20	Urban	Primary	6	Olgoasthenozoospermia
9.	Raguendra Singh	22	Rural	Primary	3	Pus cells in semen
10.	Virendra Prashar	30	Rural	Primary	8	Asthenozoospermia
11.	Raguveer Vishwakerma	25	Rural	Primary	2	H/o orichites, Exposure ATT taken
12.	Raguveer Singh	31	Rural	Primary	8	Hydrocele testicular atrophy azoospermia full of pus cells.
13.	Chander Prakash	22	Rural	Primary	5	Normal Semen analysis Urine- pus cells.
14.	Hanumat Singh	25	Rural	Primary	4	Normal samen analysis
15.	Pradeep Srivastava	30	Rural	Primary	5	Normal semen analysis
16.	Sunil	27	Urban	Primary	4	Oligoasthenozoospermia
17.	Rakesh	21	Urban	Primary	2 ½	.
18.	Phool Singh	24	Rural	Primary	8	Pus cells in semen
19.	Mewa Ram	28	Urban	Primary	6	.
20.	Bablu	25	Rural	Primary	3	.
21.	Raj Kumar	30	Urban	Primary	11	.
22.	Ramji	30	Urban	Secondary	8	Pus cells in semen
23.	Prem singh	30	Urban	Primary	5	Pus sells in semen Asthenozoospermia
24.	Ashik	32	Urban	Primary	8	Oligoasthenozoospermia
25.	Bhagwan Das	22	Urban	Primary	4	Pus cells in semen Normal
26.	Rajendra Kumar	25	Rural	Primary	2	Oligoasthenozoospermia Pus cells in urine
27.	Harcharan	20	Rural	Primary	5	Oligoasthenozoospermia with Pus cells in semen
28.	Ram Sahey	27	Rural	Primary	12	Pus cells in semen
29.	Ram Chandra	24	Rural	Secondary	6	Pus cells in semen
30.	Bhola Ram	30	Urban	Primary	8	Pus cells in semen
31.	Pushpendra	22	Urban	Primary	3	Pus cells in semen
32.	Kamlesh Saini	29	Urban	Primary	1 3/4	Pus cells in semen
33.	Durga Prasad Singh	28	Urban	Primary	6	.
34.	Dhani Ram	25	Rural	Primary	7	Normal Seman analysis
35.	Lalta Prasad	25	Rural	Primary	5	.
36.	Bhupat Singh	20	Rural	Primary	3	.

37.	Ram Das	30	Rural	Primary	10	Alb. traces in urine
38.	Ashok	27	Rural	Primary	10	.
39.	Munna Lal	28	Urban	Primary	3	.
40.	Rakesh Kumar	25	Urban	Secondary	5½	Normal
41	Kamal Kant	25	Urban	Primary	3	Asthenozoospermia with anemia
42.	Pooran	25	Rural	Secondary	7	Normal
43.	V.P. Mishra	26	Urban	Primary	2	Normal, VDRL positive
44.	Dhaniram	25	Urban	Primary	4	Pus cells in semen
45.	Virendra	38	Urban	Primary	11	Oligozoospermia
46.	Fared	29	Urban	Primary	4	Asthenozoospermia
47.	Pradeep	32	Urban	Primary	7	Normal
48.	Mehtoos Khan	28	Rural	Primary	3.5	Normal
49.	Ram Gambhir	26	Rural	Primary	2	Normal
50.	S.K. Sharma	29	Rural	Primary	5	Azoospermia
51.	Brij Mohan	26	Urban	Primary	2	Normal
52.	Kamlesh Sahu	26	Urban	Primary	2	Oligoasthenozoospermia
53.	Jamin Khan	28	Rural	Primary	4	Azoospermia
54.	Rakesh Agarwal	29	Urban	Primary	3	Azoospermia & VDRL Positive
55.	Raja Ram	30	Rural	Secondary	7	Azoospermia
56.	Badku	36	Urban	Primary	11	Normal
57.	Khalid Ahmed	24	Urban	Primary	1.5	Normal
58.	Aasha Ram	34	Rural	Secondary	10	Normal
59.	Ramesh Kumar	22	Urban	Primary	1	Azoospermia
60.	Imtiaz Khan	27	Rural	Primary	2	Normal Pus cells in semen
61.	Brijesh Verma	30	Rural	Primary	4	Not classified
62.	Halke	29	Rural	Primary	4	Oligozoospermia
63.	Aslam Mian	24	Urban	Primary	2	Oligoasthenozoospermia
64.	Ram Mohan	32	Rural	Primary	7.5	Nomal
65.	Kallu Ram	28	Urban	Primary	4	Oligozoospermia
66.	R.K. Yadav	28	Rural	Secondary	2.5	Oligoasthenozoospermia
67.	P.K. Yadav	29	Rural	Primary	6	Oligoasthenozoospermia
68.	V.P. Mishra	24	Rural	Primary	1.5	Normal Pus cells in semen
69.	Vinod	30	Rural	Primary	3	Oligoasthenozoospermia Pus cells in semen
70.	Arun Singh	28	Rural	Primary	3.5	Oligozoospermia
71.	Kamlesh	27	Rural	Primary	2	Oligoasthenozoospermia
72.	R.K. Singh	34	Rural	Primary	11	Azoospermia
73.	Sunil Gupta	29	Rural	Secondary	6	Oligoasthenozoospermia Pus cells in semen
74.	Raja Mohan	27	Rural	Primary	4.5	Oligozoospermia
75.	Mohammed	28	Rural	Primary	4	Azoospermia
76.	Prem Narayan	23	Urban	Primary	3	not classified
77.	Mahendra Singh	32	Rural	Primary	7	Azoospermia
78.	Kanhya lal	24	Urban	Primary	2	Oligozoospermia